"EXPERIMENTAL GLAUCOMA IN THE RABBIT"

A T H E S I S

SUBMITTED IN PART FULFILMENT OF

THE REQUIREMENTS

FOR THE

D E G R E E

O F

D O C T O R O F M E D I C I N E

I N

THE UNIVERSITY OF CAPE TOWN

B Y

MAURICE H. LUNTZ,

M.B.(CAPETOWN), D.O.(LOND.), D.O.M.S.(IRELAND),
F.R.C.S.ED.

AUGUST 1963

T O

J. A. R. L.

P R E F A C E

The work described in this thesis was conceived and executed by myself. I have described a technique by which chronic glaucoma can be experimentally produced in rabbits without causing any apparent ocular damage and closely resembling the disease as it occurs in man.

This has not been successfully achieved before; previous and contemporary investigators have been unable to produce glaucoma experimentally without causing severe ocular damage nor have they been able to produce anything more than a transitory ocular hypertension. My experiments have opened a new field for investigating glaucoma; it is now possible to study the natural history of this disease in an experimental animal, to observe the effects of new drugs used in glaucoma treatment before releasing them to the public

/and

and to plan experiments with the knowledge that a suitable experimental model is available.

This investigation has also served to stress the probable importance of the episclera, particularly the episcleral blood vessels, in the pathogenesis of primary open angle glaucoma.

Recently the importance of this tissue has been overshadowed by almost exclusive attention to the trabecular region although investigations attempting to demonstrate trabecular pathology in cases of open angle glaucoma have proved disappointing. The time has come that research on open angle glaucoma be broadened to include other structures as well as the trabecular tissue. The fact that chronic glaucoma, closely resembling the disease in man can be produced in rabbits by traumatising the episclera, strongly suggests that episcleral pathology may be an important

/factor

factor in the pathogenesis and aetiology of some cases of open angle glaucoma in man. There certainly is an indication for more attention to be paid to this area, a view strongly supported by the results of my research.

My experiments are divided into two series, the first undertaken in the surgical laboratories of the University of Cape Town with facilities provided by Professor J.H. Louw and Associate Professor Chris Barnard. Financial assistance came as a grant from the Foucade and C.L. Herman bequests. Technical assistance including the taking and printing of my photographs was offered by the laboratory staff.

The second series of experiments were carried out in the Nuffield Laboratory of Ophthalmology, University of Oxford. Technical assistance included photography and the preparation of slides for histology.

/I

I am indebted to the technical staff of both institutions for their aid.

My love for research and enthusiasm for the practice of Ophthalmology is attributable to four of my teachers : Messrs. Redmond Smith, A.G. Leigh and A.G. Cross, Surgeons at Moorfields Eye Hospital, London and Consultant Ophthalmologists at St. Mary's Hospital, London, and to Dr. R.L.H. Townsend, Ophthalmologist, Cape Town. To these men I owe a special debt of thanks.

Finally, I hope that my work will significantly aid the quest for new knowledge about glaucoma.

TABLE OF CONTENTS

/Section VI

TABLE OF ILLUSTRATIONS

TABLES

SECTION I

EXPERIMENTAL GLAUCOMA IN THE RABBIT

INTRODUCTION

Our knowledge and understanding of primary simple glaucoma in man has in recent years been enriched by a vast amount of research undertaken on a world-wide basis. Nevertheless there are still many unsolved problems especially as regards aetiology and natural history. Progress has been hampered because the disease cannot be satisfactorily reproduced in animals for experimental study. Sir George Pickering (1955) wrote : "a time-honoured method of investigating disease is to try and reproduce it in animals. This method has proved of the utmost value and without it the advances that have taken place all over the front of medicine would not have occurred". This statement will no doubt prove as true of glaucoma as of other diseases.

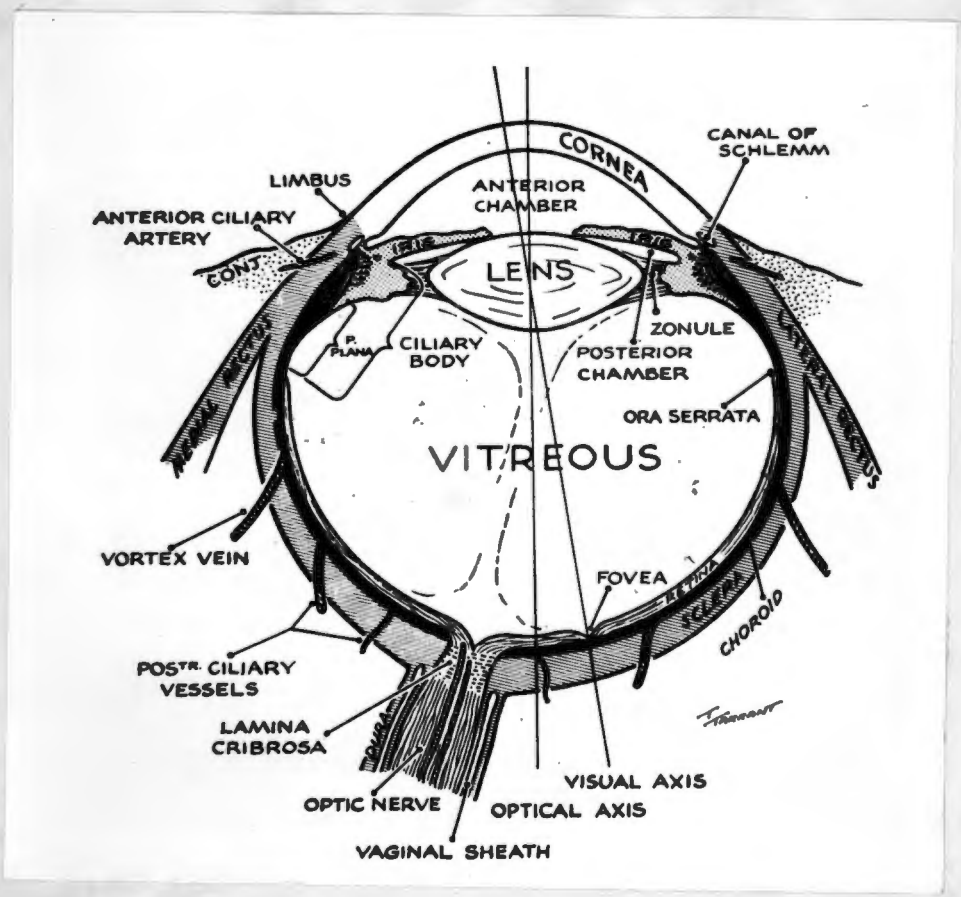
/One

One essential in any attempt to reproduce disease experimentally is that it should resemble the malady as it occurs in man. Previous workers attempting to reproduce glaucoma and bearing this in mind, have adapted the current theories for the aetiology of primary simple glaucoma in man to their techniques for reproducing it in the experimental animal. Some managed to produce raised ocular pressures over a period as long as six months, but always at the price of gross structural and functional damage to the eye. However, affected eyes in man characteristically appear to be clinically and histologically normal apart from the effects of prolonged ocular hypertension, e.g. cupping of the optic disc. What is required therefore is a technique which produces ocular hypertension in an experimental eye that is maintained over some months or longer but which does not cause any apparent structural or functional damage to the eye. Such damage

/may

FIG. 1

Diagrammatic section of an eye. The canal of Schlemm, which is situated at the angle of the anterior chamber, is indicated.



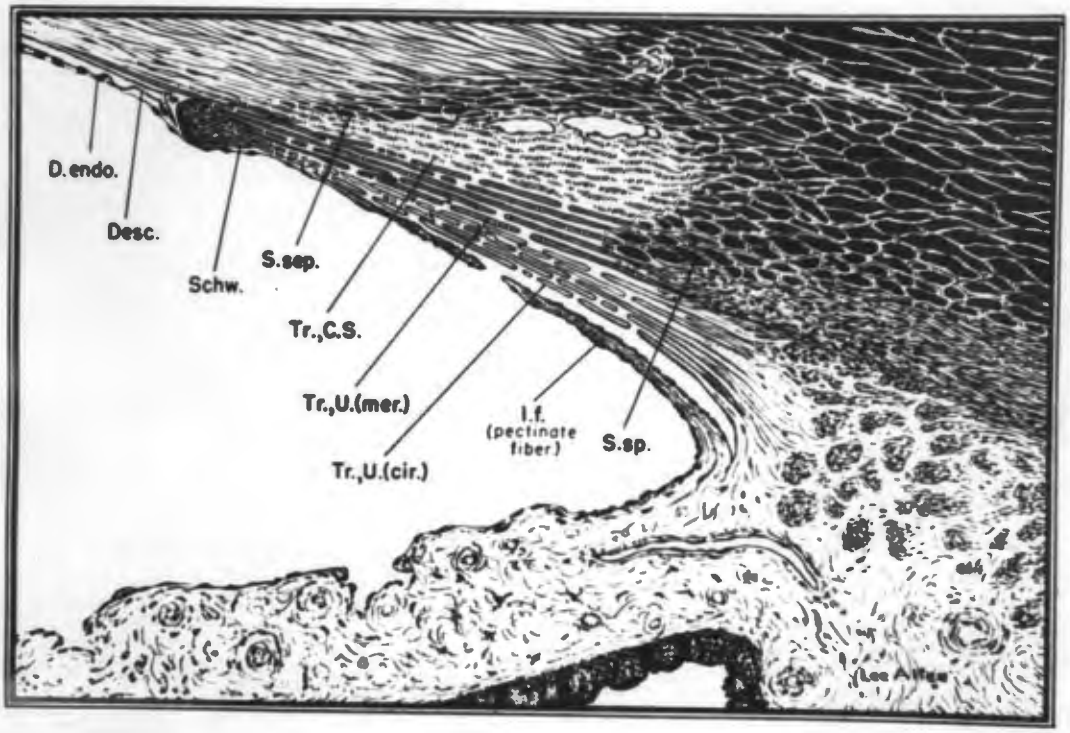
may occur later secondary to the ocular hypertension and would be permissible and indeed expected. It is the purpose of this thesis to describe such a technique and to discuss the results obtained. In the first instance I intend to discuss present concepts of the aetiology of primary simple glaucoma and the attempts by previous workers to reproduce the disease experimentally. From their experience and failures has come the necessary background for this technique.

The cause of primary simple glaucoma in man is now widely accepted as being an increased resistance to the outflow of aqueous humour at the anterior chamber angle (Fig. 1). This concept has assumed pride of place since Leber (1873) described the angle of the anterior chamber and postulated that aqueous drainage took place mainly at this site. This was confirmed by Priestley Smith (1891) in a brilliant series of perfusion experiments on

/enucleated

FIG. 2

Diagram of a high power view of the anterior chamber angle showing iris, ciliary body, trabecular tissue and cornea-scleral junction. The canal of Schlemm is visible as two venous channels on the corneal side of the cornea-scleral trabeculum.



KEY

D. endo - Corneal endotheleum.
Desc - Descemets membrane.
Schw. - Schwalbe's line.
S. Sep. - Scleral septum.
Tr., C.S. - Corneal portion of the trabecular tissue.
Tr., U (mer.) - Uveal fibres (meridional) of the trabecular tissue.
Tr., U (cir.) - Uveal fibres (circular) of the trabecular tissue.
I.f. - Pectinate fibre - Pectinate tissue.
S. sp. - Scleral spur.

enucleated eyes. He went further and stressed that obstruction at the angle of the anterior chamber was primarily responsible for raised ocular tension. Gonioscopy has shown that acute closed angle glaucoma occurs when the iris obstructs the entry of aqueous into the angle.

The work of Priestley Smith in particular focussed attention on the angle of the anterior chamber. Formed by the junction of the cornea and the iris root, it is bounded by the cornea and the cornea-scleral junction anteriorly and the iris root and ciliary body posteriorly (Fig. 2). Situated in the angle is the canal of Schlemm which is a venous channel into which the aqueous drains. First described by Schlemm (1831) it is related distally to sclera and proximally to the scleral spur and the trabecular tissue, which will be described in more detail later.

A large volume of work began to

/accumulate

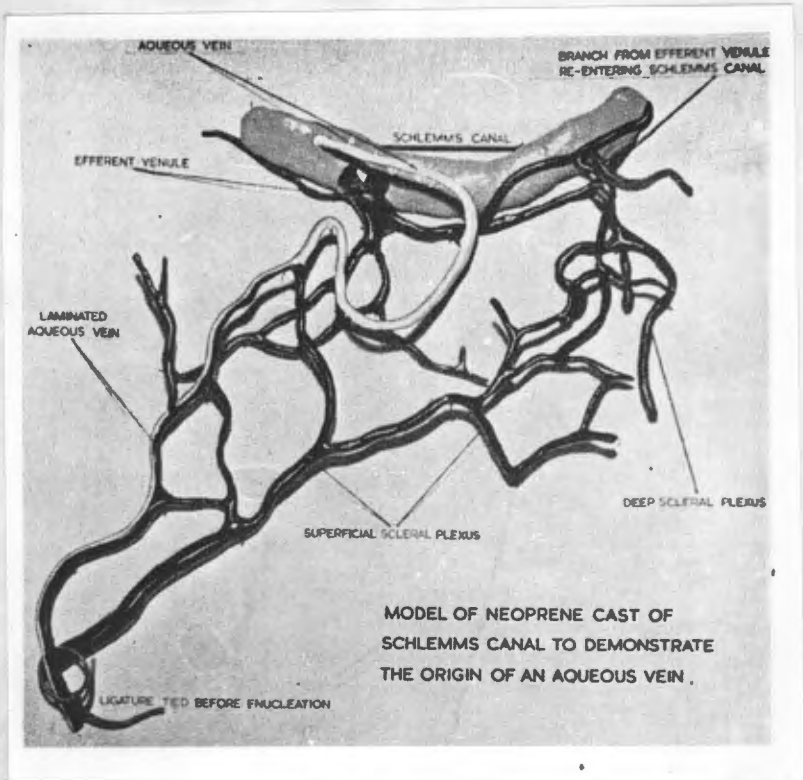
accumulate on the anatomy of this structure in an attempt to find, by comparison with the normal, some histological or anatomical abnormality of this area in cases of primary simple glaucoma. No abnormality has been conclusively demonstrated.

The early studies were made upon specimens injected with dyes or Indian ink, reconstructed serial sections or combinations of these two methods. These workers were able to describe the minute anatomy of the perilimbal circulation accurately and in detail, adequately reviewed by Dvorak-Theobald (1934). Later studies were made by Ashton (1951-1952) using a more sophisticated technique by means of which he was able to confirm what had been previously taught. He filled Schlemm's canal, the intrascleral and episcleral venous plexus's with neoprene and then digested the ocular tissues with pepsin and trypsin so that a neoprene cast of the perilimbal circulation

/remained

FIG. 3

Model of neoprene cast of Schlemm's canal showing an aqueous vein, the deep scleral and superficial scleral plexus's.



remained. By tying the aqueous veins in the episclera with a tantalum wire loop before removing the eye, these could be identified and studied in the neoprene cast.

A neoprene cast of the perilimbal circulation which demonstrates an aqueous vein tied with a tantalum wire loop is shown (Fig. 3).

Ashton confirmed what previous workers had postulated. One of the best and most detailed descriptions of the anatomy of Schlemm's canal and its exit channels was given by Maggiore (1917). His conclusions were based on serial sections done with meticulous care, reconstructing the anatomy with infinite patience. It is a striking tribute that recent work has added nothing of importance to his description of this region.

These investigations made it clear that the aqueous humour from Schlemm's canal

/drained

drained into the episcleral venous plexus by a series of intercommunicating plexus's in the sclera and episclera, eventually reaching the superficial vessels of the episclera. Approximately 30 external collector channels, irregularly distributed and varying in size and shape, conveyed aqueous from Schlemm's canal to the deep scleral plexus which in turn anastomosed with the intrascleral plexus, the aqueous finally reaching the episcleral venous plexus where it was absorbed into the general circulation.

Ascher (1951, 1952) and Goldmann (1946) independently described vessels in the episclera which contained either aqueous alone or a column of blood side by side with one of aqueous depending on the relationship between the pressure in the episcleral veins and in the canal of Schlemm. These vessels are called 'aqueous veins'. Ashton (1951, 1952) using his neoprene cast technique showed that

/aqueous

aqueous veins arose either from Schlemm's canal directly or in a more indirect fashion via the deep scleral plexus and then travelled directly to join the episcleral veins without any anastomosis in the sclera. They are an anatomical peculiarity present in approximately 90 per cent of the population, but have no other significance. They can be seen biomicroscopically as they traverse the episclera.

That portion of the angle situated between the anterior chamber and Schlemm's canal is composed mainly of modified cornea and called trabecular tissue. Posterior to this are the scleral spur and the anterior portion of the ciliary body. It is through the trabecular tissue that the aqueous gains access to Schlemm's canal.

The anatomy of this area was beautifully described by Salzmann (1912) using the

/histological

histological methods available at the time. Recently Speakman (1960) using more sophisticated methods and also electron microscopy has confirmed Salzmann's work. He described it as modified corneal and uveal tissue composed of numerous flat collagen "plates" arranged as a meshwork and separated from one another to allow aqueous to percolate through. Each plate contains a central collagen cone enclosed by an elastic membrane (possibly modified Descemet's membrane) and surrounded by endothelial cells similar to the corneal endothelium. He divided the trabecular tissue into a uveal portion on the inner aspect derived embryologically from uvea and a corneal portion adjacent to Schlemm's canal, which is embryologically part of the cornea.

Aqueous drainage from the anterior chamber is considered to take place predominantly at the angle of the anterior chamber of the eye via Schlemm's canal. The aqueous

/gains

gains access to Schlemm's canal by percolating through the trabecular tissue and leaves Schlemm's canal mainly through collector channels reaching the episcleral venous plexus and the venous circulation by way of the deep scleral and intrascleral plexus's. A limited proportion of this drainage from Schlemm's canal reaches the episcleral venous plexus directly via aqueous veins which by-pass the deep scleral and intrascleral plexus's.

Considering the anatomy of the anterior chamber angle in the light of present day knowledge, it is evident that obstruction to the aqueous outflow in primary simple glaucoma in man could occur at one of three sites :

a) the trabecular tissue, immediately proximal to Schlemm's canal,

b) at Schlemm's canal (rare),

c) the outflow channels from Schlemm's canal of which the episcleral venous plexus forms the ultimate stage.

SECTION II

PATHOGENESIS OF PRIMARY SIMPLE GLAUCOMA

At present it is generally held that the obstruction is in the trabecular tissue and is due to either sclerosis or degeneration of this tissue. The evidence for this is flimsy and at best inadequate. It is based on the work of Grant (1958), Barkan (1954) and Huggert (1957). Grant did a perfusion experiment on an enucleated glaucomatous eye, and showed that the resistance to outflow could be significantly reduced by rupturing the trabecular tissue with a sharp pointed needle. This experiment followed on a previous report of a similar experiment (Grant 1955) which gave the opposite result.

Barkan claimed that he was able to see an abnormal veil-like membrane over the trabecular tissue when gonioscoping eyes with primary simple glaucoma. Other investigators have been unable to confirm this observation

/in

in spite of the fact that gonioscopy is a routine clinical procedure in the investigation of these cases. Huggert (1957) showed that glaucoma could be reproduced in rabbits by introducing particles of varying sizes into the aqueous and thereby blocking the trabecular 'pores'.

On the other hand the importance of the episcleral plexus in the drainage mechanism is recognized but has never assumed clinical importance as an aetiological factor in primary simple glaucoma, probably because histological or clinical abnormalities of this tissue have never been demonstrated.

Thomassen (1948) showed that changes in the pressure of episcleral blood vessels anticipated changes in the intraocular pressure in the glaucomatous eye.

He measured intraocular pressure with a Schiötz tonometer and the episcleral venous

/pressure

pressure with a water manometer. Water was conducted from the manometer to a small transparent chamber covered on one side with a thin cellophane paper. The chamber was placed on the vein to be examined while an assistant lifted the water container, thus increasing the pressure, until the vein was seen to collapse. The pressure was read on the manometer. He concluded that, because the pressure in the aqueous veins is proportional to the intraocular pressure, the same relationship must exist between these veins and the episcleral blood veins; therefore when the intraocular pressure is in a rising phase, the pressure in the blood veins will be high in proportion to the aqueous veins, and the outflow through the latter will accordingly be hampered. This idea was confirmed by Bain (1954) who used a more refined technique to measure changes in the pressure of the episcleral veins. In studying a case with a

normal and glaucomatous eye, however, he found that the changes in venous pressure in both eyes were the same. He concluded that changes in the venous pressure do not themselves cause pathological rises in ocular tension, but may play an important role in eyes with an already defective drainage. Thus while confirming Thomassen's results, he disagreed with his hypothesis.

The work of Dobree (1953) is also of interest. He studied the changes in the calibre of the episcleral arteries, veins and capillaries during the diurnal fluctuations in tension and recorded them photographically. He found in both normal and glaucomatous eyes that where the rise in ocular tension was moderate in degree, there was an association between the vascular tone in the episcleral vessels (mainly the venous side) and the ocular tension. Thus when the latter was raised, the former were in a phase of relative

/vasoconstriction

vasoconstriction, while the period of most marked vascularity coincided with the lowest pressure found. He suggested that these changes facilitated the outflow of aqueous by causing a reduction in pressure in the venous outflow from the canal of Schlemm.

There is therefore a close relationship between the episcleral venous pressure and the intraocular pressure in man. This can be shown experimentally as well.

Huggert (1951) produced a rise in intraocular pressure in 25 out of 33 normal and glaucomatous eyes by means of a contact lens with a narrow haptic, and Ascher (1951) reported that aqueous outflow may be blocked or at least retarded by contact lenses, even if they rested gently on the conjunctival surface.

On the clinical side Fuchs (1923) reported that corrosive injuries, especially those affecting the limbus, quite frequently caused

/a

a rise in ocular tension which developed several days later and might last a week or more. He also pointed out that in scleritis glaucoma might supervene through ectasia of the sclera. Luntz and Redmond Smith (1960) reported four Negro patients with primary simple glaucoma who at operation had a pathological degree of fibrosis of the episclera. Ten Negro patients operated on for pterygeum were used as a control series. It was suggested that obliteration of episcleral veins due to fibrosis may constitute a specific type of glaucoma in this race. This would also explain why it has always been difficult to achieve a satisfactory drainage operation in these people. Friedenwald (1950) in discussing reasons for the failure of glaucoma operations remarked that post-operative histological studies showed more episcleral scarring in Negroes than in white patients.

/To .

To summarise it can be said that although the consensus of present-day opinion asserts that the maximum resistance to aqueous outflow in primary simple glaucoma is in the trabecular tissue, there is a body of circumstantial evidence which suggests that in some cases the maximum resistance to outflow may be found in the episclera. This should be considered as an alternative aetiological factor in cases of primary simple glaucoma.

SECTION III

EARLY ATTEMPTS AT PRODUCING GLAUCOMA EXPERIMENTALLY

These concepts are not new and it is not surprising that the earliest attempts at reproducing glaucoma in animals were directed at obliterating the episcleral venous plexus. Schöler (1879) and Heisrath (1879) heat cauterized the whole area around the limbus and the latter also attempted painting the area with silver nitrate. Bentzen (1895) repeated the latter experiment.

The discovery of aqueous veins on the surface of the eye prompted Weekers and Prigott (1950) to attempt selective diathermy cauterization of these vessels.

The ingenuity of previous workers did not stop at the episclera. Koster (1895) showed that raised intraocular pressure could be obtained for short periods of time by

/ligating

ligating the vortex veins. Weber (1877) managed to block the filtration angle by adding suspensions of oil to the aqueous while Bentzen (1895) used suspensions of bacteria. Milton Flocks, Tsukahara and Miller (1959) achieved raised intraocular pressures for about three weeks in one eye in each of 49 animals by the application of a tight encircling rubber band sewn to the sclera around the equator of the eye and under the extraocular muscles.

Rabbits were the most widely used animals in these experiments. In those of Milton Flocks et al monkeys and cats were used also but were found to be unsuitable. All these efforts failed in two important respects :

i) raised intraocular pressure was achieved only for a short time, usually a few days,

ii) the technique used caused gross

/structural

structural and functional damage to the treated eye which rapidly atrophied.

In an excellent paper in 1957, Huggert reported a series of experiments in which he produced raised intraocular pressure in rabbits, using methods that adapted the principles elaborated by earlier investigators. He obstructed aqueous outflow in one of three ways :

a) by diathermic cautery of the episcleral aqueous veins near the limbus corneae. Aqueous-containing vessels were recognized by injecting fluorescein under pressure into the anterior chamber and examining the episcleral vessels with short-wave light under a conjunctival flap. These were then cauterized paying attention to the vessels running along the muscles. After adequate cauterization he observed that the flow still proceeded through intrascleral and

/possibly

possibly choroidal vessels to the posterior pole. This important observation will be more fully discussed later. Twenty-two eyes were treated in this way.

b) By ligating the trunks of the vessels at the posterior pole of the eye. The vessels and muscles in this area were ligated with silk sutures and the ligatures adjusted to obstruct the venous outflow but not arterial inflow. Ten eyes were treated by this method.

c) By blocking the filtration angle with suspensions of particles of a relatively uniform size and of gradually increasing dimensions. The materials were Thorotrast approximately 0.1 μ, Polystrene 0.26 μ, polymethyl methacrylate approximately 0.5 μ and larger bacterial particles. Injection into the anterior chamber was done under pressure to force the particles into the angle. Fourteen

days were allowed to elapse between each injection into the same eye, particles being used in a sequence of increasing size. He found that particles 0.75 μ in size and over produced a rise in ocular tension for two to three weeks, whereas less than 0.75 μ did not. Ten eyes were treated in this manner. Huggert was unable to satisfy the requirements I cited for reproducing glaucoma experimentally. He achieved raised intraocular pressures of two to three weeks duration only, apart from a few cases treated by ligating the vortex veins. Such a drastic procedure, however, would cause severe damage to the eye and embarrassment to function. Unfortunately the final appearance of these eyes is not described.

Whilst reviewing these previous attempts at reproducing glaucoma experimentally, it seemed to me that fibrosis of the episcleral

/plexus

plexus offered the best means of achieving a raised intraocular pressure with the least amount of ocular damage. Added to this was the thought that disease of the episclera in man might play a greater role in the aetiology of glaucoma than was generally recognized and it would be interesting to know whether fibrosis of this tissue could result in glaucoma. One knew that diathermy cautery of the episcleral aqueous veins caused a raised intraocular pressure which was transient (Huggert 1957); was this the result of too violent a reaction with destruction of the ciliary body resulting in hypotony after the initial rise in pressure? I adopted this argument and decided to try a less violent medium for achieving episcleral fibrosis through chemical cautery. This, if the correct sclerosing agent could be found, would result in a less violent reaction spread over a longer period of time, and could be easily

/repeated

repeated. I was fortunate enough to find this agent at my second attempt.

SECTION IV

PRESENT EXPERIMENTS - MATERIALS AND EXPERIMENTAL TECHNIQUE

MATERIALS

Twelve grey female rabbits were used because they are more docile and easier to handle than males. Six were bred from the stock at the Cape Town University Medical School, which are not a pure breed and are closest to pure-bred Chinchilla's. A second series of six were Chinchilla rabbits obtained at Oxford University. Rabbits are docile animals, have relatively large eyes and corneae and submit uncomplainingly to tonometry. Histologically the rabbit anterior chamber angle has structural differences to that of man. These were well described by Davis (1929). Essentially these differences occurred in the meshwork which was better developed in the rabbit. The collagenous

/fibres

fibres were much finer and not enclosed by elastic fibres as in man. They ran as isolated fibres. Schlemm's canal was not a single channel, as in man, but rather a series of channels forming a plexus. In spite of these anatomical differences Ruskell (1961) showed that there was close functional similarity to man, which justifies its use as an experimental animal. Using the neoprene cast technique as described by Ashton (1951) he showed that in rabbits the major outflow of aqueous was to the episcleral vessels, as in man.

His results also supported the idea of a secondary route for aqueous outflow as advocated by Nemetz (1956), from the filtration angle through the intrascleral plexus to the choroid. These connections had also been demonstrated in man by Ashton (1955). There is considerable disagreement about the extent to which this route could be used in rabbits

/under

under physiological and pathological conditions. The fact that prolonged ocular hypertension can be produced by blocking the episcleral outflow strongly implies that this route is of minor importance as an alternative outflow channel in the breed of rabbit used in these experiments. It is generally agreed that this is also the case in man.

The rabbit, then, has an aqueous drainage system which is similar to that in man, apart from some minor anatomical differences. Monkeys have an angle very similar to that in man but unfortunately it is impossible to do tonometries on them with local anaesthetic.

Another argument in favour of using rabbits was that normal rabbits produced fairly constant intraocular pressure readings with repeated indentation tonometries, using local anaesthetic. The average tension in 28 rabbit eyes was found by Becker and Constant

(1956) to be 18 mm.Hg. with a standard deviation of ± 3.7. They used a Mueller electronic tonometer and took their measurements in vivo. This was repeated whilst doing tonographies in vitro and found to be 19 mm.Hg. with a standard deviation of ± 4.8, a remarkably close agreement. Kornblüth and Linnér (1955), and Becker and Constant (1956) showed that tonography could be applied to the rabbit eye with valid results. The latter did a series on twenty-eight rabbit eyes in vivo and found an average facility of outflow "C" of 0.33 (standard deviation ± 0.08). These eyes were then perfused with isotonic saline in vivo and an average C value of 0.34 obtained (standard deviation ± 0.10). When perfused in vitro, the average C value was 0.37 (standard deviation ± 0.11). Considering the experimental errors inherent in tonography, the C values obtained by all methods are surprisingly similar.

/SCLEROSING

SCLEROSING MATERIALS :

The rabbits were given subconjunctival injections of a sclerosing fluid, using either a 0.175% solution of silver nitrate, a 1% solution of Ethanolamide (Ethanolamine Oleate) or a 5% solution of Phenol in almond oil. The latter two preparations are standard sclerosing agents used in the treatment of varicosities e.g. in the rectum. Of these only the Phenol solution proved to be satisfactory, causing a rise of intraocular pressure but no apparent macroscopic or microscopic damage to the eye.

The silver nitrate was prepared by the hospital dispensary (Groote Schuur Hospital). The Ethanolamide and Phenol came as commercial preparations in 5 c.c. ampoules; the former prepared by May and Baker (S.A.) Pty. Limited, Port Elizabeth; the latter by Glaxo-Allenbury (S.A.) Pty. Limited, Durban.

/DESCRIPTION

DESCRIPTION OF EXPERIMENTAL TECHNIQUE

a) TONOMETRY :

The animal was tied in a duster so that only its head and neck protruded. Both eyes were anaesthetized using two drops topically of a 1% solution of Tetracaine in aqueous. The technician then placed the animal on his knee and held its head with the right cornea facing vertically upwards. The tonometer (a standardized Schiötz tonometer) was cleaned with spirit and checked on a hard convex surface, supplied with the instrument : the pointer should be deflected to the zero position on the calibrated scale. If correct the instrument was placed vertically on the cornea, the operator carefully holding the eyelids apart with one hand, being careful not to press on the globe, and the reading on the calibrated scale noted. This was recorded in millimetres of mercury by consulting

/Friedenwald's

Friedenwald's nomogram (1957). Two readings were made, one using a 5.5 gm. weight and another using a 7.5 gm. weight.

Occasionally the nictitating membrane gave trouble by covering the cornea, overcome by patiently waiting for it to retract and then proceeding.

Having measured the right intraocular pressure, the same procedure was repeated on the left eye.

The intraocular pressure was recorded in each eye of every rabbit on at least three occasions before the first subconjunctival injection was given and at weekly intervals thereafter until the experiment was terminated. Where subsequent tonometries coincided with a subconjunctival injection, the tonometry was done first. Intraocular pressures in the first series are followed graphically in figures 8 to 12 and the initial

/subconjunctival

subconjunctival injection is indicated. Figures 13 to 17 are graphs of the intraocular pressures in the second series. These also indicate all the subconjunctival injections and also the intraocular pressures before the initial injection.

b) THE SUBCONJUNCTIVAL INJECTION :

The method of injection was the approved technique for a subconjunctival injection, the aim being to deliver the sclerosing fluid into the episcleral tissues. Two minims of sclerosing fluid was drawn up into a standard 2 c.c. syringe fitted with a No. 1 needle. The needle was then removed and a No. 18 needle fitted to the syringe.

The animal was immobilized and anaesthetized in the same way as described for tonometry and the initial intraocular pressure measurement made. A

/conjunctival

conjunctival speculum was now used to hold the lids apart, the operator fixated the conjunctiva in the desired quadrant with a pair of Lister's forceps and the No. 18 needle was pushed through the conjunctiva into episcleral tissue. One minim of the sclerosing fluid was injected, the needle was withdrawn and the speculum removed. If more than one quadrant was to be injected the procedure was repeated in each quadrant at the same sitting. There was no after-treatment. With only one exception (rabbit No. 3) one eye in each rabbit was given a subconjunctival injection of sclerosing fluid (the "treated" eye) whilst the other eye was left as a control (the "control" eye). Hence treated and control eyes could be compared in the same animal.

Work on the first series of rabbits was done in the department of surgery,

/University

University of Cape Town. These were numbered 1 to 6. The first two animals were given subconjunctival injections of the 0.175% solution of silver nitrate. This resulted in a severe local reaction, the method was abandoned and the rabbits withdrawn from the experiment.

In the remaining rabbits in this series one eye was injected subconjunctivally with the 5% Phenol in almond oil. At first one quadrant of the globe, then two and then three were injected; this failed to cause a rise in intraocular pressure. Finally all four quadrants were injected, using four minims of fluid. Positive results were obtained at this stage.

In rabbit No. 3 both eyes were injected subconjunctivally with 5% Phenol in almond oil (four quadrants) : a similar injection but of almond oil was given in

/the

the control eye of rabbit No. 4. The control eyes of the remaining rabbits in this series were left untreated.

These injections were repeated at regular intervals at two and again at four weeks after the initial injection.

c) TREATMENT OF THE GLAUCOMATOUS EYES IN THE FIRST SERIES :

This part of the experiment was terminated after some weeks by sacrificing the rabbits and removing their eyes for histological study. Before doing this an iris inclusion operation was done in some eyes; in other rabbits Pilocarpine nitrate drops in 1% - 4% solution, was given together with Acetazolamide (Diamox) 125 mgs. daily by mouth in tablet form. These are established methods of treating ocular hypertension in man and the effect on the

/intraocular

intraocular pressure in these glaucomatous rabbit eyes was recorded. Although the purpose of this thesis is to describe a method of reproducing chronic glaucoma in rabbits, the results of treating their glaucomatous eyes offers an interesting digression and has useful research possibilities. The results of treatment and a discussion of its possible research applications is presented later.

The iris inclusion was performed by an ab externo technique. The animal was anaesthetized by the open-mask ether method and one attempted to control the depth of anaesthesia at a level just deep enough for the operative procedure. The anaesthetized animal was placed on its side with the eye to be operated on uppermost.

The operator scrubbed and donned sterile gown, cap, mask and gloves. The

rabbit was dressed in sterile towels so that only the eye to be operated on was exposed. A conjunctival speculum was inserted and a few drops of 1% Tetracaine instilled topically.

A limbal-based conjunctival flap was fashioned and retracted over the cornea. A scleral incision was made with a Bard-Parker knife and No. 11 blade into the anterior chamber, 1 millimetre from the limbus at the 12 o'clock position. The iris prolapsed through this incision and was pulled out until the pupillary border became visible. A vertical incision was then made with de Wecker's scissors from the pupillary edge to the root of the iris. The iris was torn from its root for a short distance on either side of this incision, fashioning two iris pillars. These were left passing through the scleral incision at each edge and the

/conjunctiva

conjunctiva sutured in place over them with 6 - 0 catgut sutures.

A few drops of Chloramphenicol 1% were instilled into the conjunctival sac and an eye pad applied which was removed immediately the animal regained consciousness.

Within a few days the conjunctiva had healed and a bleb of aqueous had formed under it.

The second series of six rabbits was treated by a similar experimental technique at the Nuffield Laboratory of Ophthalmology, University of Oxford. In five of these the subconjunctival injection contained Phenol in almond oil as before. In one 4 minims of the Ethanolamide solution was injected. This resulted in a violent local reaction with sloughing of the sclera. The rabbit was

/discarded

discarded, leaving five. These five were numbered 2(a) to 6(a). The subconjunctival injections were repeated but at varying intervals (see Fig. 13 to 17). The intraocular pressure was measured by tonometry. They were not given treatment as in the first series, but were subjected to inflow and outflow studies. Outflow studies were done by a four minute tonography; inflow studies by the fluorescein appearance time technique. These studies were done some time after the subconjunctival injections when the intraocular pressure in the treated eyes was raised.

d) TONOGRAPHY :

The Mueller tonometer was allowed to warm up for at least half an hour. The tonometer head and plunger was scrupulously cleaned with spirit and the scale was calibrated in the usual way to zero

/and

and 7, using a 5.5 gm. weight on the tonometer head. The reading on the recorder scale was checked against the tonometer scale and adjusted to coincide with this, and the recorder was checked to ensure that it traced properly.

The animal was immobilized and anaesthetized in the same way as described for tonometry. The tonometer head was placed on the cornea of the eye to be measured and the reading begun. A four minute tonography was the rule in each eye. During this period the animal had to remain perfectly quiet and this was best achieved in a closed, isolated room free from outside interferences. Drugs were not used. If the animal moved or the tracing was unsatisfactory it had to be repeated. Because absolute isolation in a hospital is almost impossible to achieve the tonometer was adjusted to optimum

/sensitivity

sensitivity so that the recording showed the respiratory waves but they remained of small amplitude in most cases. These readings were subsequently photographed for presentation with this thesis (Figs. 18 to 27).

e) FLUORESCEIN APPEARANCE TIME :

A sterile 10% solution of fluorescein in normal saline was prepared by the Oxford Eye Hospital pharmacist. The rabbit to be investigated was given a few drops of a mixture of Cyclogyl 1% (Cyclopentolate Hydrochloride) and Phenylephrine 10% into one conjunctival sac. Half an hour later when the pupil was maximally dilated the animal was bound in a duster with the head and neck protruding and was placed in position in front of a slit-lamp. Its head was held with the dilated pupil facing the lamp by a

/technician

technician who also operated a stop-watch. One ear had already been shaved to expose a vein.

Five cubic centimetres of the fluorescein solution was drawn up into a 5 c.c. luer-lock syringe with a No. 1. needle. A No. 20. needle was fitted and the fluorescein injected intravenously into the prepared ear vein as rapidly as possible. As soon as the injection was started the stop-watch was released and the eye observed under slit-lamp magnification using the low power. The end point occurred when the fluorescein was seen at the edge of the pupil, easily recognized by its green colour; the time taken between the injection of fluorescein intravenously and the end point was recorded as the fluorescein appearance time. (Table II, page 97).

The two eyes of each rabbit were

/investigated

investigated on separate occasions as it was impossible for one observer to visualize both eyes simultaneously through the slit-lamp. An identical technique was used each time and the measurement was done twice in each eye.

SECTION V

ANALYSIS OF METHODS USED FOR MEASURING INTRAOCULAR PRESSURE AND AQUEOUS DYNAMICS.

The intraocular pressure was measured by tonometry and the facility of aqueous outflow assessed by tonography. Aqueous inflow was measured by the fluorescein appearance time test.

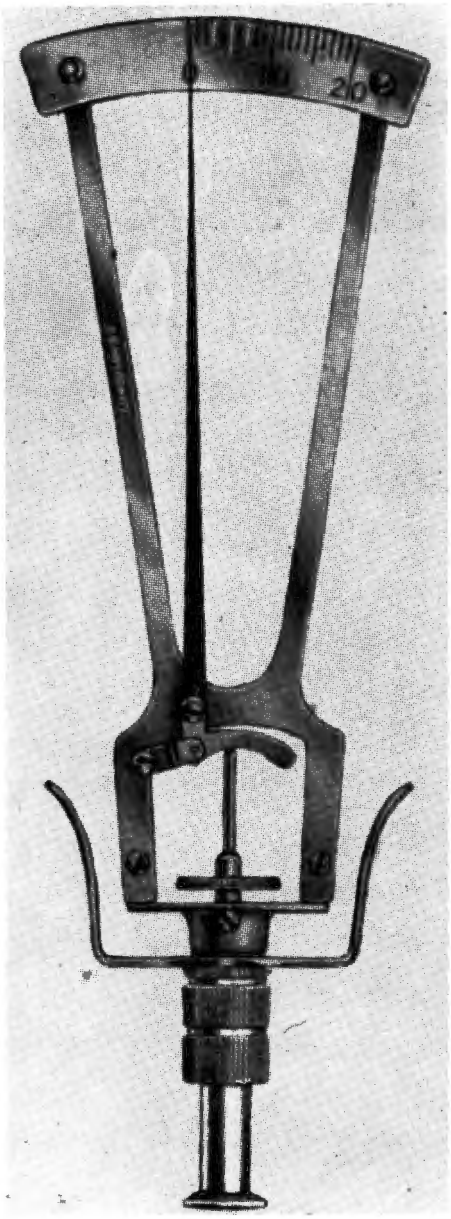
TONOMETRY :

This method was used in preference to direct manometry of the anterior chamber. Although the latter is the more accurate method it is not practicable to do repeated manometries on a rabbit eye. In a study of this kind where gross changes of intraocular pressure were being recorded in the treated eyes and compared to control eyes in the same animal, absolute accuracy in measuring ocular tension was not essential. Evidence has been presented that indentation tonometry gives

/constant

FIG. 4

A Schiötz tonometer with the 5.5 Gm. weight ready for use.



constant and fairly accurate results in rabbit eyes. As an additional precaution, two weights were used for each measurement (5.5 gm. and 7.5 gm.) and the average recorded. The 1957 Friedenwald nomograms were consulted to convert scale readings to millimetres of mercury pressure. A standardized, weighted and recently calibrated Schiötz tonometer, manufactured by Winter and Co., West Germany, was used (Fig. 4).

The impression tonometer measures the depth of impression made by a plunger of standard size and weight, either 5.5 gm., 7.5 gm., 10 gm. or 15 gm., acting on the cornea. The depth of impression produced is magnified by a system of levers and measured by deflection of a pointer on a calibrated scale. The pointer is deflected either mechanically, as in the Schiötz tonometer, or electronically, as in the Mueller electronic tonometer. In the latter instrument the metal plunger fits

/into

into the holder which contains a magnetic coil. Movement of the plunger in this holder produces a galvanic current which is fed into the machine and deflects the pointer. This value represents the amount of corneal indentation produced by the plunger which is dependent not only on the intraocular pressure but also on the resistance of the outer coats of the eye, which are relatively indistensible, and the resistance of the intraocular fluids to displacement. The tonometer scale reading represents the intraocular pressure in the eye after indentation (this value is referred to as Pt), but what one requires to know is the intraocular pressure in the eye before indentation (the Po value).

This is calculated from the Pt value and expressed as millimetres of mercury.

There are therefore two sources of error inherent in the use of an impression tonometer.

a) IN MEASURING Pt :

/Apart

Apart from sources of error in the mechanical design of the tonometer, which can be avoided by using a properly calibrated instrument, the major difficulty is that one is measuring not only intraocular pressure but also the resistance of the coats of the eye to distension and of the ocular fluids to displacement. Friedenwald coined the term "ocular rigidity" to cover these two factors.

Thus the Pt value will depend on the ocular rigidity in that eye as this affects the degree of indentation produced by the tonometer plunger.

The Pt value is read directly from the tonometer scale which is converted into the Po value as millimetres of mercury pressure by consulting a nomogram. This was constructed by Friedenwald (1954, 1957) from a tonometer calibrated on a perfused enucleated human eye. The

/pressure

pressure in this eye could be varied at will and the tonometer scale suitably calibrated.

CALIBRATION OF THE TONOMETER :

The perfused eye was connected by a polythene tube to a reservoir with a tap interposed. The reservoir in turn was connected to a water manometer which could be set at the required pressure. The true Po value (i.e. the intraocular pressure before indentation of the eye) could be obtained directly by filling the eye to a given pressure and then closing the tap before placing the tonometer on the globe (the closed stop-cock method).

Unfortunately this method gave variable results. Friedenwald was forced to resort to an open stop-cock method leaving the tap open and the eye in communication with the manometer, thus measuring Pt (the

/intraocular

intraocular pressure after indentation).

In order to minimise the effect of scleral rigidity, calibration was done on many normal eyes and the average position of the pointer for each intraocular pressure value taken as the tonometer scale reading for that pressure value. There was close agreement in eyes with average scleral rigidity and only these were used in the calibration.

The next step involved the calculation of Po from Pt and the expression of the Po value in millimetres of mercury. The final result is reflected in the Friedenwald nomograms.

This calculation provides the second possible source of error.

b) CALCULATION OF Po FROM Pt :

The Po and Pt values are linked by

/a

a formula, based on Friedenwald's demonstration that for an eye of given size the volume varies in proportion to the change of pressure (Friedenwald 1937).

$$K = \frac{\log Pt - \log Po}{Vc} \quad \ldots\ldots\ldots\ldots (1)$$

where K = coefficient of ocular rigidity,

Pt = intraocular pressure after indentation of the cornea by the tonometer plunger,

Po = intraocular pressure before indentation of the cornea by the tonometer plunger,

Vc = volume of corneal indentation.

The unknowns in the formula, apart from Po, were K and Vc. Friedenwald obtained these values by modifying the technique he used for calibrating the indentation tonometer by the open stop-cock method. When a tonometer was placed on the eye, fluid was displaced into the

mercury manometer and it is evident that the volume of fluid so displaced was equivalent to Vc. Two different weights were used (5.5 gm. and 7.5 gm.) and the two values so obtained were applied to the formula

$$K = \frac{\log Pt_2 - \log Pt_1}{Vc_2 - Vc_1} \quad \ldots\ldots\ldots (2)$$

where K = coefficient ocular rigidity,

Pt_2 = intraocular pressure after corneal indentation with a 7.5 gm. weight,

Pt_1 = intraocular pressure after corneal indentation with a 5.5 gm. weight,

Vc_2 = volume of fluid displaced by indentation with a 7.5 gm. weight,

Vc_1 = volume of fluid displaced by indentation with a 5.5 gm. weight.

/By

By varying the pressure manometer and so the Pt value, Friedenwald constructed a table linking Pt with the corresponding volume of fluid displaced (i.e. he found the Vc values corresponding to different Pt values).

From Formula (2) he was able to calculate K. By calculating this value in each of a number of normal eyes, he was able to derive an average normal value for K of 0.0215. In doing this calculation, he assumed that the initial volume in each eye tested was approximately the same. His K values actually varied from 0.005 to 0.060, and the figure of 0.0215 represented an average value.

Recently Sampson and Girard (1961) have criticized Friedenwald's assumption of a roughly constant initial volume in the eyes he tested. They maintain that this may vary considerably and if taken

/into

into account the K values in different eyes will vary over a much smaller range.

They base their criticism on the physical law that in hollow, water-filled rubber spheres, a given change in volume produced by indentation or other means, will result in a change in pressure which is an inverse function of the initial volume of the sphere.

These authors, using the same technique as Friedenwald for measuring the Pt and Vc values, express the volume increment for each Pt value as a percentage of the initial volume of the eye. This they calculated by measuring two diameters of the eye and applying the formula $V = 2 \pi r^2$. They claim that by using this method scleral rigidity remains constant for practically all eyes, thereby eliminating a major source of error. This is an interesting challenge but

/requires

requires confirmation. There is a possible error in measuring the diameters of the eye and the calculation of initial volume from the formula $V = 2\pi r^2$, which assumes that the eye is a perfect sphere.

By substituting the values for Vc and Pt obtained by measurement and the calculated average K values in formula (1), Friedenwald was able to calculate the Po value for each corresponding Pt value and, expressing this in millimetres of mercury, constructed his nomograms for each of the different weights (5.5 gm., 7.5 gm., 10 gm. and 15 gm.) of the Schiötz tonometer. These are the nomograms that have been used in this thesis to convert the Schiötz scale readings into millimetres of mercury.

It is at once apparent that the

/major

major source of error lies in the assumption of an average value for K when calculating Po. Where an eye has a K value which is not average, the nomogram will give an incorrect assessment of the intraocular pressure. Fortunately the scleral rigidity for an individual eye can be easily checked by comparing the intraocular pressures from the nomogram with two different weights on the tonometer. If the eye has an average K value the Po result using both weights will be similar. If the K value is not average they will differ by more than 3 millimetres of mercury.

Rabbits used in these experiments were selected so that their intraocular pressure readings with a 5.5 gm. weight and 7.5 gm. weight were within 3 millimetres of mercury of one another. By ensuring that control and treated eyes were compared in the same animal

/and

and that all the eyes used had average K values the tonometer was used at its maximum efficiency. Scrupulous care was taken to keep the tonometer plunger clean and free from mucous.

TONOGRAPHY :

Tonography was used to measure the facility of aqueous outflow in the control and treated eyes. The technique was devised by Grant (1950) and in principle measured the displacement of aqueous from an eye by a weighted tonometer plunger resting on the cornea for 4 minutes. The coefficient of the facility of aqueous humour outflow (C) was calculated from the change in ocular volume. This value would depend on the resistance to aqueous outflow at the anterior chamber angle. One would expect, and indeed Grant found, that in glaucoma the C value was lower than in the normal, indicating that in glaucomatous eyes the

/resistance

FIG. 5

The Mueller electronic tonometer (above) with the Honeywell-Brown recorder (below). On the left is the fixation lamp and on the right a tray with anaesthetic drops, spirits and swabs.

resistance to aqueous outflow is greater than in normal eyes. The C value in normal eyes was found to vary from 0.15 to 0.60 with an average value of 0.33. Grant and subsequent workers used an electronic indentation tonometer with a galvanic recorder. The recorder was essential not only to provide a graphic record of the test, but also to demonstrate that the test had been technically satisfactory. In such cases one obtained a tracing with well marked respiratory and pulse waves and showing a gentle declivity.

The tonographic tracings in this series were performed with a Mueller electronic tonometer (manufactured by V. Mueller & Company, Chicago), coupled to a Honeywell-Brown recorder (manufactured by Honeywell Controls Limited, Ruislip Road east, Greenford, Middlesex) (Fig. 5).

THEORETICAL CONSIDERATIONS :

/The

The test depends on the decrease in ocular volume produced over the 4 minute period. This can be measured in the same way as described for tonometry.

With this information the C value is calculated each time the test is done from the formula

$$C = \frac{\Delta V}{t(\text{Ave Pt} - \text{Po})} \quad \ldots\ldots\ldots (3)$$

where ΔV = loss of volume from eye during time of the test t,

Ave Pt = average intraocular pressure over 4 minutes excluding the first reading (i.e. open manometer pressure),

Po = first reading (closed manometer pressure),

t = time,

C = outflow in cubic μ litres per minute per millimetre of mercury pressure gradient (facility of aqueous outflow).

/The

The C values for different Pt and Po values have been calculated and in practice one reads this value directly from Friedenwald's nomogram.

Knowing the value of C one can calculate

a) resistance to outflow = 1/C and

b) steady state flow rate (F) i.e. outflow in cubic μ litres per minute of aqueous from the anterior chamber.

$F = C(Po-Pv) = 2.2\ \mu$ litres per minute in normal human eyes,

where Pv = episcleral venous pressure, measured as 10 mm.Hg. in normal eyes. It is assumed that this is the same for glaucomatous eyes, possibly a wrong assumption.

Thus the sources of error in this test are to be found in

a) variations in ocular rigidity which affects the value of V and Po;

b) variations in the episcleral venous pressure which directly influences the rate

/of

of outflow and therefore the 'Ave Pt' value.

In spite of these sources of error, it has been clearly shown that tonography is a useful clinical and experimental method of estimating the resistance to aqueous humour outflow from the eye. Grant, in his original paper describing the technique in 1950, showed that the change in ocular volume during tonography was a function of the resistance to aqueous outflow and was not the result of inhibition of aqueous formation or due to the expression of blood from the vascular bed of the eye. Using normal rabbit eyes in living and dead animals he demonstrated that the tonographic decline was greater in dead eyes, where aqueous was not being produced and where the vascular bed had collapsed, than in the eyes of living animals. When he perfused these dead eyes with saline solution at a pressure of 25 millimetres of mercury he could reproduce the normal curves for a living eye,

/and

and by substituting methyl cellulose solution these were strikingly altered, both in dead and living eyes. In enucleated human eyes, devoid of a circulation and a mechanism for aqueous humour formation, he found the tonographic curves were similar to those in normal eyes in a living person and he concluded therefore, that the tonography measurement reflected principally the outflow of aqueous humour.

His tests also showed that consistent results were obtained when measuring the same eye repeatedly both in humans and rabbits.

Kornblüth and Linnér (1955) presented convincing evidence that tonography could be applied equally well to rabbit eyes. Tonographic measurements were made with a Mueller electronic tonometer and 5.5 gm. and 10 gm. weights on 20 rabbit eyes. They calculated a value of 0.015 for the average scleral rigidity, a figure slightly lower than that for

/the

the human eye. This meant that the estimate of Po using human calibration tables should be too low. However, when comparing the final Pt and Po values using human calibration tables after tonographic tracings on normal rabbit eyes were continued until no further fall in pressure took place, indicating that a steady state had been reached, they found close agreement on these values, so that the difference in average scleral rigidity did not require correction when applying human calibration tables to the rabbit. In the steady state, where aqueous formation and outflow are equal, the intraocular pressure with the tonometer resting on the eye (Pt) should be approximately the same as it was in the undisturbed eye before the tonometer was placed on the cornea (Po).

The episcleral venous pressure was measured in 14 normal rabbit eyes and found to be 8.94 $\pm$ 0.17 mm.Hg., giving a fairly constant

/value

value in good agreement with that found in human eyes (10 mm.Hg.).

Using Friedenwald's calibration tables these authors performed tonographies on 14 normal rabbit eyes and calculated the average C values for the right and left eye separately.

The coefficient was found to be the same in the two eyes and unrelated to the level of the original intraocular pressure. They found an average C value of 0.30 with a standard deviation of ± 0.042 in the right eyes and ± 0.040 in the left eyes. The standard error of the mean was 0.011 in both eyes.

This observation was an important one because it meant that the technique could be used for experimental studies in the rabbit.

Grant and Trotter (1955) had gone a long way to prove the validity of tonography, when they showed, using cannulated, enucleated

/human

human and animal eyes, that the average normal facility of outflow from enucleated human eyes (31 eyes) was similar to the average normal facility of outflow found clinically by tonography and the use of Friedenwald's tables (0.27 as compared with 0.233). But they did not use the same eyes in the two methods. In the eyes that were cannulated they determined the facility of aqueous outflow in two ways : in the first, they calculated scleral rigidity from Friedenwald's formula by measuring the degree of pressure change induced in an eye by the injection of a known volume of fluid (0.9% sodium chloride);

$$\text{scleral rigidity} = \frac{\log \frac{P_1}{P_2}}{\Delta V} \quad \ldots\ldots\ldots\ldots (4)$$

where P_1 = initial intraocular pressure

P_2 = final intraocular pressure

ΔV = volume of fluid introduced into the eye.

/The

The facility of aqueous outflow was now determined by discontinuing the inflow of fluid and keeping a strain gauge connected to the eye. The pressure was found to fall rapidly along a straight line, F. A portion of line F, several minutes long, was selected and pressure changes (F1 and F2) recorded. The volume of fluid flowing out of the eye could now be calculated from the formula :

$$\Delta V = \frac{\frac{\log F1}{\log F2} + 0.1\,(F1-F2)}{\text{Scleral rigidity}} \quad \ldots\ldots (5)$$

The coefficient of outflow was obtained by utilizing the known relationship between rate of outflow and intraocular pressure :

$$\frac{\Delta V}{(T)\quad(\text{Ave } P)} \quad \ldots\ldots\ldots\ldots (6)$$

where T = time over which measurement was taken

Ave P = average intraocular pressure between F1 and F2

/At

At the same time they showed that the standard electronic tonometer used in the measurements, when calibrated in the usual way by open and closed stop-cock methods against the standard Schiötz tonometer on enucleated eyes, gave accurate readings. The second method was to calculate the outflow facility with the eye in a steady state when the intraocular pressure was constant and outflow equalled inflow. Ocular volume, therefore, was constant, and no consideration of scleral rigidity was necessary. They found that with the eye connected to the inflow system a steady state was reached given sufficient time, irrespective of whether the inflow was initially greater than the outflow or vice versa. The inflow system was precalibrated (by accurate weighing of effluent) so that the relationship between the rate of flow (effluent) and the pressure gradient of the inflowing fluid (the difference between the intraocular pressure and the height of the

/fluid

fluid in the inflow reservoir) was established. The coefficient of facility of outflow (C) was calculated from the steady state intraocular pressure (p) and rate of outflow (k) :

$$C = \frac{k}{p} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (7).$$

The two methods gave results which were in satisfactory agreement, the first method giving an average facility of outflow of 0.177 μ litres per minute per millimetre of mercury, and the second, which was independent of the influence of scleral rigidity, a value of 0.178 μ litres per minute per millimetre of mercury, both at room temperature. The average individual disparity between the two methods was 0.027 μ litres. This compared well with the average value of 0.233 μ litres per minute per millimetre of mercury at body temperature (approximately 0.18 at room temperature) obtained by tonography on 171 normal eyes in vivo by the same authors.

/The

The close similarity in the average outflow facilities in normal eyes obtained by three different methods is strong testimony for the validity of tonographic results.

Becker and Constant (1956) using rabbits and local anaesthetic were able to compare the results of tonography in vitro and in vivo using the same eye for each method. In addition they were able to control the experiment because they calculated the Po and C values directly by cannulating and perfusing the same eye prior to enucleation and after enucleation. Perfusion was carried out at pressures varying from 0 mm.Hg. to 40 mm.Hg. and the aqueous inflow was measured as the rate of inflow in μ litres per minute at various pressure levels (Pl).

The C value was then calculated from the formula :

$$C = \frac{I}{Pl - Po} \quad \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

/where

where I = rate of saline inflow

Pl = inflow pressure (mm.Hg.)

Po = intraocular pressure before cannulation (mm.Hg.).

The results obtained by these methods once again showed a striking similarity and once again the C value is shown to be independent of the intraocular pressure in the undisturbed eye (Po).

He found the average intraocular pressure in the normal rabbit eye was 19 mm.Hg. with a standard deviation of ± 4.8. The average C value :

a) by perfusion in vivo, was 0.34 with a s.d. of ± 0.10,

b) by perfusion in vitro, was 0.37 with a s.d. of ± 0.11,

c) by tonography in vivo, was 0.33 with a s.d. of 0.09.

These results are similar to those of

/Kornblüth

Kornblüth and Linnér which were discussed earlier.

Becker went one stage further and repeated the experiment in four human eyes about to be enucleated. Once again the values he obtained were reasonably similar and consistent. The tonography values varied from 0.20 to 0.27, the perfusion in vivo from 0.21 to 0.26, and the perfusion in vitro from 0.23 to 0.29.

Tuovinen (1961) analysed the results of treatment in 361 patients with primary glaucoma attending the glaucoma clinic of the University of Helsinki. He found a close relationship between the severity of the disease and the facility of aqueous humour outflow, (C). Successful treatment depended on the establishment of an adequate C value.

Although each of these investigations taken singly as a test for the validity of

/tonography

tonography is not conclusive, taken as a whole they form a considerable weight of evidence that, used clinically as a means of measuring aqueous outflow facility, it provides consistent and meaningful values; also that it can be used in the rabbit eye with Friedenwald's tables, and this was the method used in my work.

FLUORESCEIN APPEARANCE TIME :

The appearance time of fluorescein in the anterior chamber has been used as an index of aqueous inflow. Seidel (1918), Weekers (1921) and Kajigaya (1951) demonstrated that it enters the anterior chamber either through the anterior surface of the iris or through the ciliary body or both. Linnér and Friedenwald (1957) using rabbits studied the time interval which elapsed between the dye reaching the eye (nictitating membrane) after intravenous injection and appearing in the pupillary area. When changing the rate of

/aqueous

flow experimentally with the carbonic anhydrase inhibitor Acetazolamide, they found that this time interval was significantly prolonged. They concluded that it was a useful measure of the rate of aqueous flow. Berggren (1956) studied the relation between log-dose of the dye and log-time of appearance of the dye in the pupil, and found a straight line if the appearance time was less than five minutes, suggesting that when doing this test a suitable concentration of dye would be such that it appeared in the pupil in under five minutes.

There is a major uncertainty in this method, because it is not possible clearly to differentiate between fluorescein coming from the anterior chamber (e.g. from the anterior surface of the iris) and that coming from the posterior chamber. Coming from the anterior chamber it would be seen at once, from the posterior chamber only when it reached the

pupillary margin travelling by diffusion and convection currents along the posterior surface of the iris (Berggren 1956), the time taken depending on the size of the pupil. With this in mind the test was carried out with the pupil dilated so that fluorescein from the posterior chamber had less distance to cover to reach the pupillary margin and pupil size remained constant for all the eyes tested. It was considered that using this minor modification, large differences in the fluorescein appearance time where the two eyes of the same animal were being compared and where the appearance time was less than five minutes, could be accepted as a significant difference in the rate of aqueous flow in the two eyes.

SECTION VI

FIG. 6

Photograph of a control rabbit eye.

FIG. 7

A treated rabbit eye (Phenol 5% in almond oil). The conjunctiva is slightly suffused.

R E S U L T S

A) APPEARANCE OF TREATED EYES :-

Immediately following the subconjunctival injection they were red and congested but no flare or cells were present when examined on the slit-lamp. They settled within a few days, the external appearance being relatively normal. This is evident from figures 6 and 7 where a control and treated eye are illustrated respectively. The conjunctiva remained freely mobile. There was no cupping of the optic discs, the fundi remaining normal in appearance.

B) INTRAOCULAR PRESSURE :-

i) FIRST SERIES :

This is presented graphically (Figs. 8 - 12), the tension recorded at

/weekly

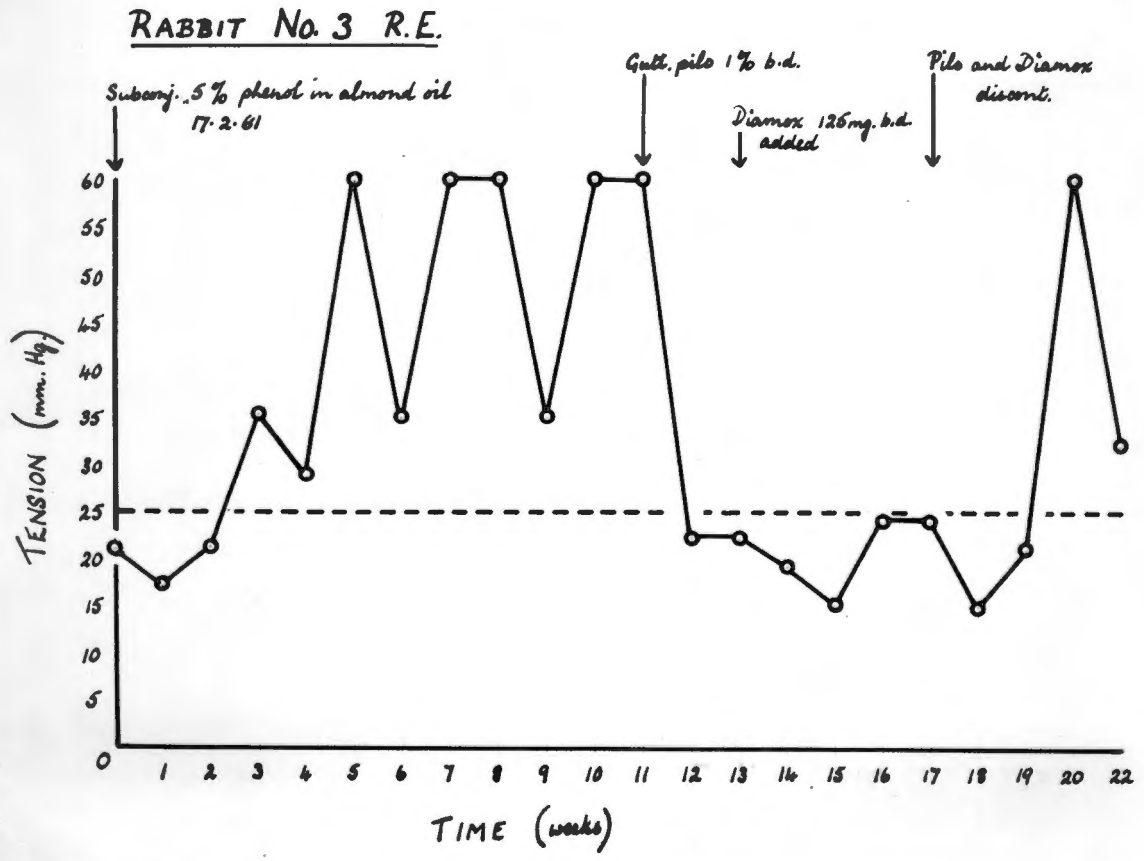
weekly intervals and plotted on the ordinate, the time in weeks along the abscissa.

In all the treated eyes except number 4, the intraocular pressure rose steadily, with some fluctuations, to 60 mm.Hg. where the graph flattens. The average time taken to reach this level was 7.5 weeks. This period represents an adjustment of ocular dynamics to the steadily increasing outflow resistance, a consequence presumably of slowly progressive episcleral fibrosis (or episcleral vascular sclerosis). As outflow resistance increases (demonstrated by the tonography records), so aqueous formation and inflow decreases (shown by the fluorescein appearance time test). Ultimately compensation fails and the intraocular pressure rises. The level of ocular hypertension attained and the rapidity

/with

FIG. 8

Intraocular pressure of right eye of rabbit No. 3 plotted against time in weeks. The timing of initial subconjunctival injection is indicated. The stippled line is of no significance. Three measurements of intraocular pressure were made before the initial subconjunctival injection, but only the third is plotted.



with which it is reached would depend on the degree of episcleral pathology and the availability of other routes for aqueous drainage from the eye (e.g. the demonstration by Ruskell and Nemetz of an alternative route to the choroidal vessels and thence to the vorticose veins). This is probably why the treated eye in rabbit number 4 did not become glaucomatous.

RABBIT NUMBER 3 :-

a) RIGHT EYE (FIG. 8) :

Base-line ocular tension before treatment with Phenol 5% in almond oil was 21 mm.Hg. The first four-quadrant subconjunctival injection was given on the 27 February 1961, the second on 3 March 1961, and the third, 17 March 1961. Ocular tension rose gradually reaching 60 mm.Hg. after five weeks and then fluctuated

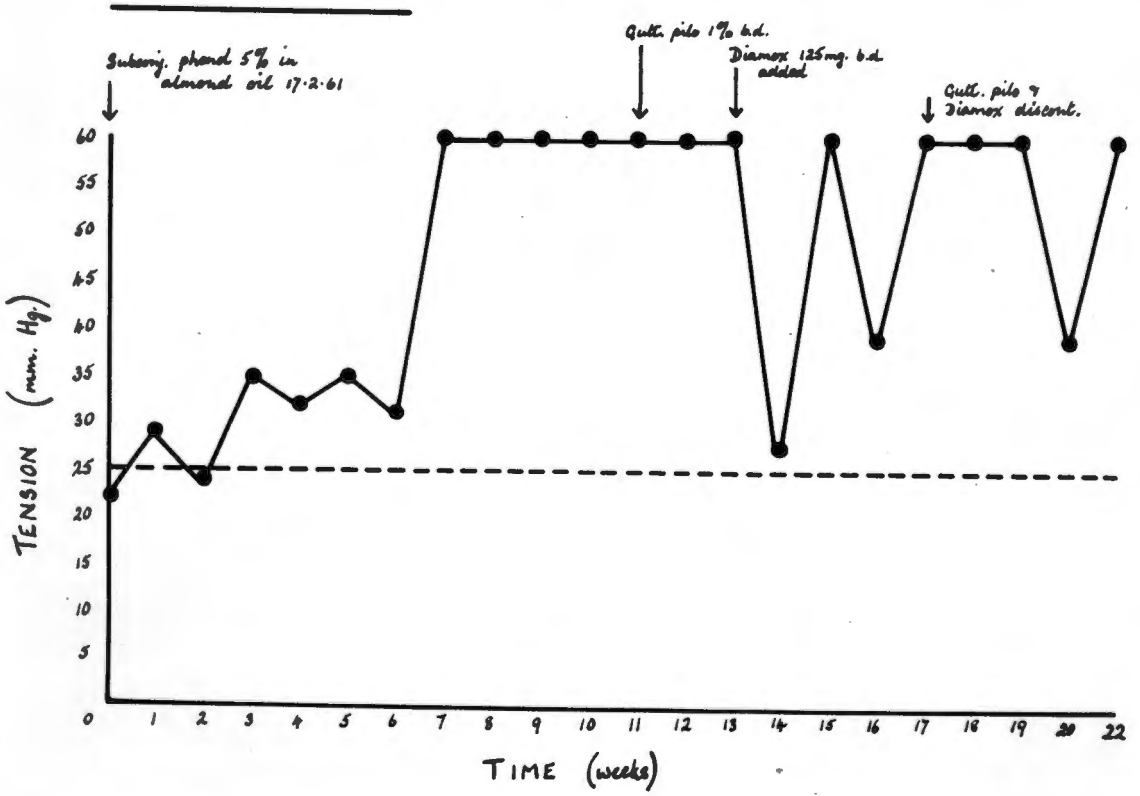
/between

between 35 mm.Hg. and 60 mm.Hg. over the following seven weeks. At this stage Guttae Pilocarpine nitrate 1% b.d. was applied topically. This had no effect and was increased to 4% b.d. with an immediate fall in tension to 22 mm.Hg. Two weeks later Diamox (acetazolamide) 125 mgm. b.d. was given by mouth. Ocular tension fell further, to 15 mm.Hg. for two weeks and then rose gradually to 24 mm.Hg. Medication was stopped after seven weeks and ocular tension returned to 60 mm.Hg. It is noteworthy that there was a latent period of two weeks without drugs during which the intraocular pressure remained at normal levels, before starting to rise. I am at a loss to explain this. A cumulative effect of Diamox, which could explain it, has never been demonstrated in the rabbit and does not occur in man. Little is known of the effects of Pilocarpine nitrate drops

/on

FIG. 9

The intraocular pressure of the left eye of rabbit No. 3 plotted against time in weeks. The initial subconjunctival injection is indicated. The stippled line is of no significance. Only the third of three measurements of intraocular pressure before the initial subconjunctival injection is plotted.



on the rabbit eye.

b) LEFT EYE (FIG. 9) :

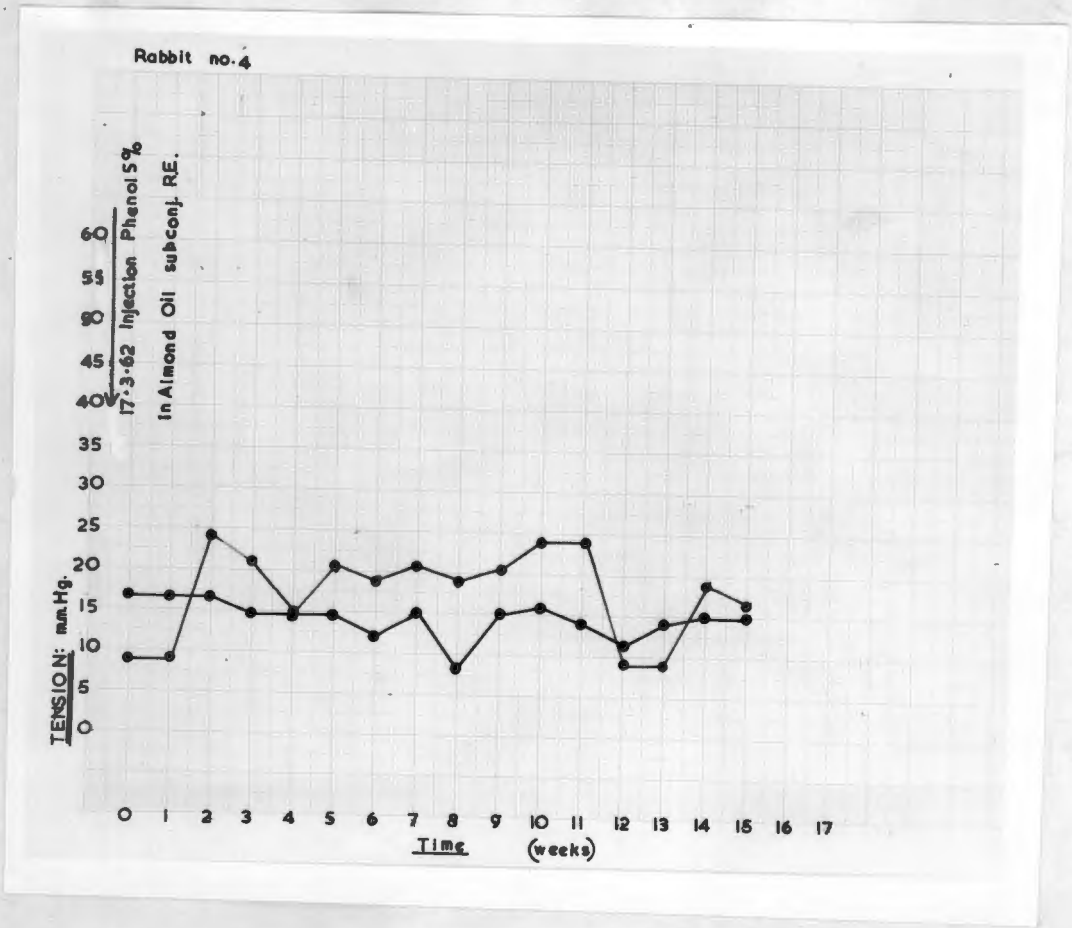
Base-line ocular tension before treatment with Phenol 5% in almond oil was 21 mm.Hg. The first four-quadrant injection was given on 17 February 1961, the second on 3 March 1961 and the third on 17 March 1961. Ocular tension rose gradually reaching 60 mm.Hg. after five weeks and then fluctuated between 35 mm. Hg. and 60 mm.Hg. over the following weeks.

Guttae Pilocarpine nitrate 1% b.d. was administered at this stage, but as it had no effect this was increased to 4% b. d., still without any effect. Diamox 125 mgm. b.d. by mouth was added and the tension dropped immediately to 27 mm.Hg., but a week later was back to 60 mm.Hg. and subsequently remained about 30 mm.Hg.

/until

FIG. 10

The intraocular pressure in both eyes of rabbit No. 4 is plotted against time in weeks. The initial subconjunctival injection given in the right eye is indicated. This eye did not become glaucomatous. The third of three pressure readings made before the initial subconjunctival injection is plotted as the first reading.



until the drugs were discontinued two weeks later. Presumably outflow resistance was greater in the left eye than in the right eye.

RABBIT NUMBER 4 (FIG. 10) :-

a) RIGHT EYE :

Phenol 5% in almond oil was injected into all four quadrants on 17 March 1961, on 31 March 1961 and on 14 April 1961. Ocular tension fluctuated between 12 mm.Hg. and 27 mm.Hg. This was the only treated eye in the series which failed to become hypertensive, possibly because of a well developed choroidal aqueous outflow route (Nemetz, Ruskell).

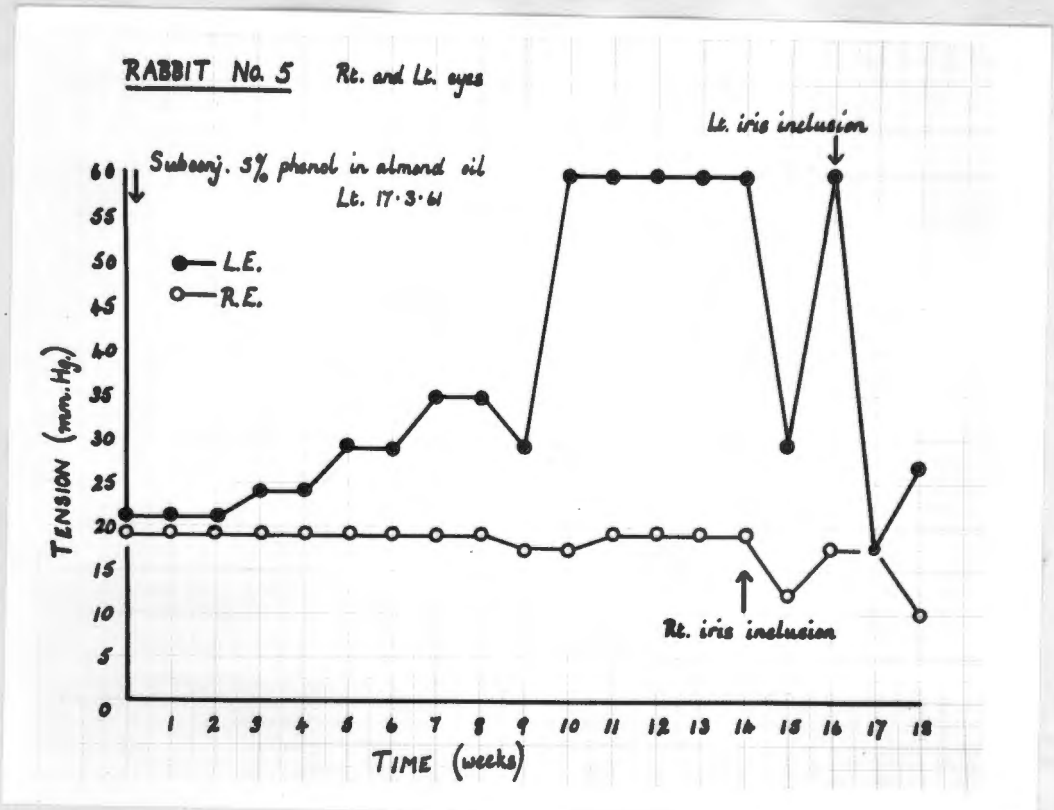
b) LEFT EYE :

This was a control eye and received subconjunctival injections of almond oil only, on 17 March 1961 and the next two

/at

FIG. 11

The intraocular pressure in rabbit No. 5 plotted against time in weeks. The initial subconjunctival injection into the left eye is indicated. The right eye is the control. The first reading plotted is the third of three measurements of the intraocular pressure made before giving the initial subconjunctival injection.



at fortnightly intervals. Intraocular pressure fluctuated between 12 mm.Hg. and 23 mm.Hg.

RABBIT NUMBER 5 (FIG. 11) :-

a) RIGHT EYE :

This was used as a control eye. The ocular tension remained constant at 19 mm.Hg. except on two occasions when it was recorded as 18 mm.Hg.

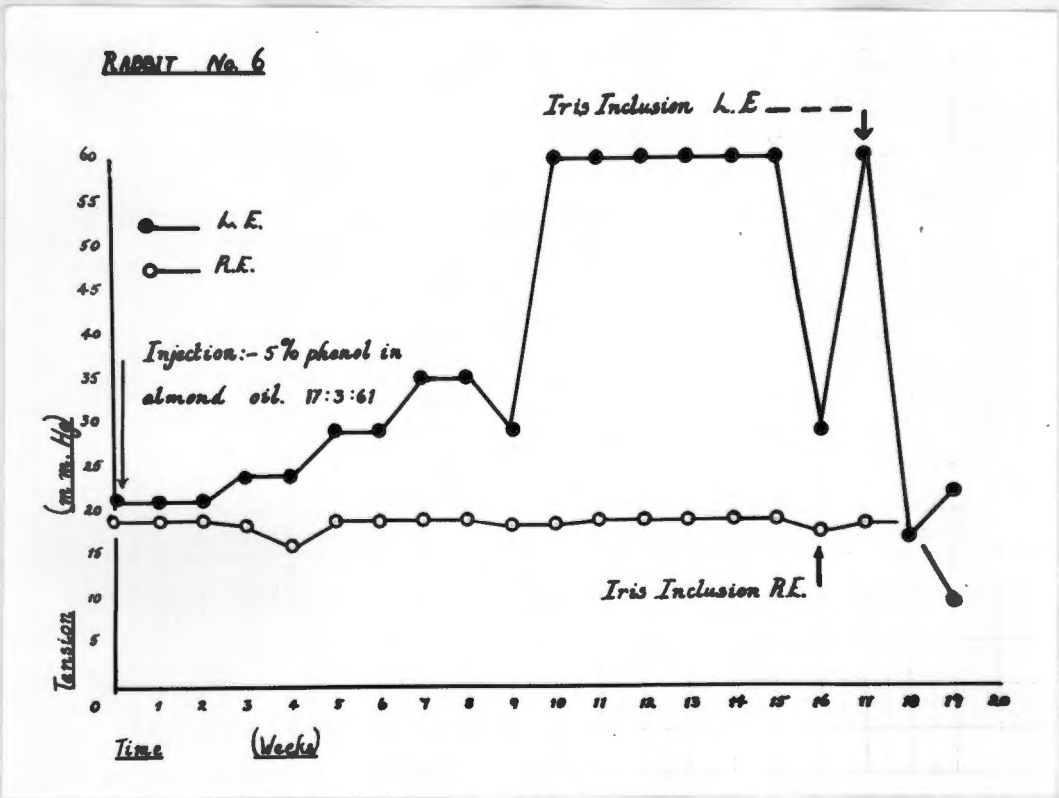
b) LEFT EYE :

The first injection of Phenol in almond oil in all four quadrants was given on the 17 March 1961 and the next two at fortnightly intervals. The base-line ocular tension was 20.5 mm.Hg. and this rose over ten weeks to 60 mm.Hg., remaining at this level for four weeks. At this point an iris inclusion done on the control eye resulted in a fall of

/intraocular

FIG. 12

Intraocular pressure of rabbit No. 6 plotted against time in weeks. The initial subconjunctival injection in the left eye is indicated. The right eye is the control. The first plotted reading is the third of three measurements of intraocular pressure made before giving the initial subconjunctival injection.



intraocular pressure in the operated eye as well as in the left eye, the latter subsequently returning to 60 mm.Hg. At the sixteenth week an iris inclusion was done in this eye and the ocular tension dropped to normal values.

RABBIT NUMBER 6 (FIG. 12) :-

a) RIGHT EYE :

This was the control eye. Intraocular pressure remained at 19 mm.Hg. with minor fluctuations except on two occasions, one of these at the sixteenth week, the result of an iris inclusion.

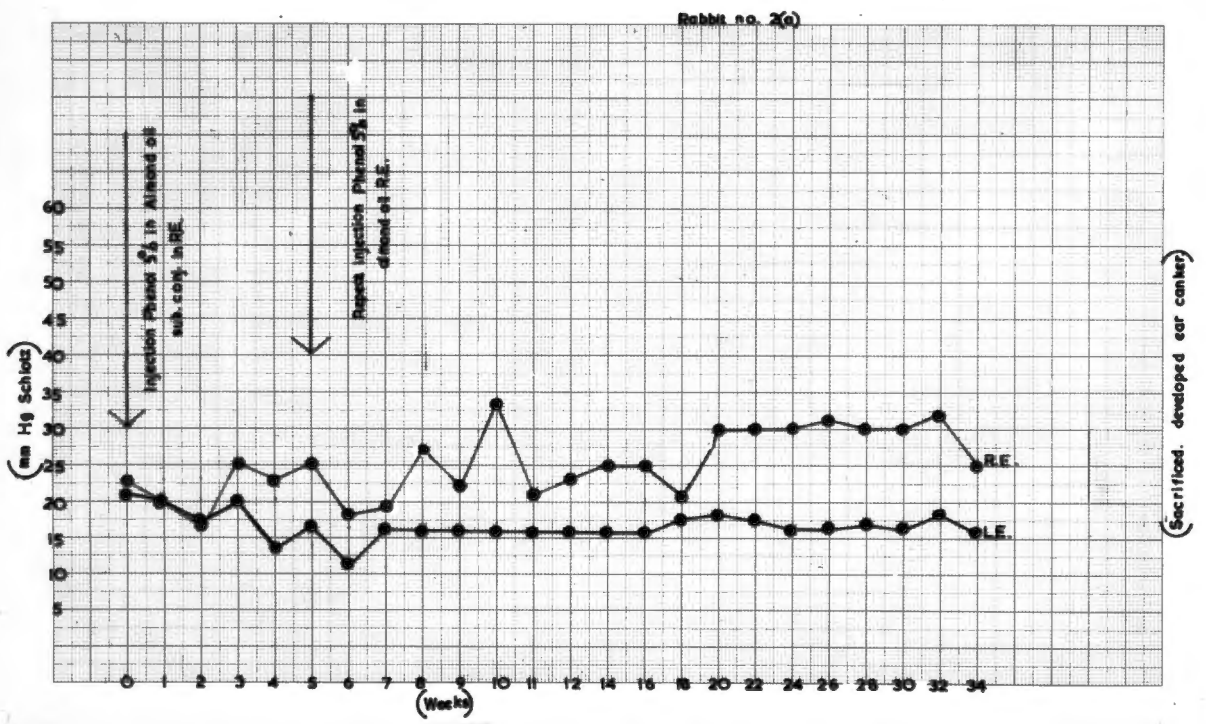
b) LEFT EYE :

The first injection of Phenol in almond oil in all four quadrants was given on the 17 March 1961, and repeated twice at fortnightly intervals. This eye reacted in an identical way to the

/left

FIG. 13

Intraocular pressures in rabbit No. 2(a) plotted against time in weeks. The timing of the two subconjunctival injections in the right eye is indicated. The left eye is the control.



left eye in rabbit number 5. There was a fall in the intraocular pressure in this eye at the sixteenth week in sympathy with a fall in pressure in the right eye following an iris inclusion. This is an interesting phenomenon, well illustrated in these two eyes.

ii) SECOND SERIES :

RABBIT NUMBER 2(a) (FIG. 13) :

a) RIGHT EYE :

This was the control eye. Intraocular pressure at the start of the experiment was 21 mm.Hg. but fell within a few weeks to 16 mm.Hg. and remained fairly constant for the 34 weeks of the test.

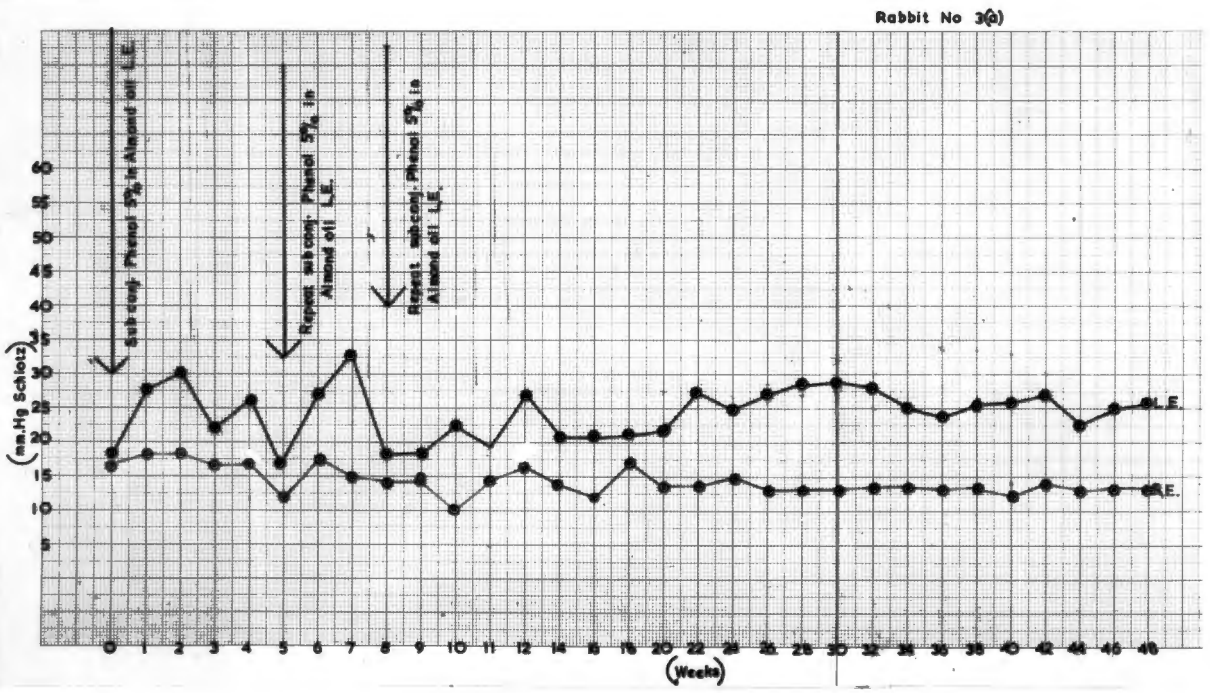
b) LEFT EYE :

Phenol in almond oil was injected on 22 September 1961 and repeated at the

/5th

FIG. 14

Intraocular pressure in rabbit No. 3(a) plotted against time in weeks. Three sub-conjunctival injections were given in the left eye and this is indicated in the figure. The right eye is the control.



5th week. Intraocular pressure was consistently higher than in the left eye, fluctuating between 22 mm.Hg. and 33 mm. Hg., from a base-line intraocular pressure of 22 mm.Hg. The rabbit developed ear canker and had to be sacrificed at the 34th week.

RABBIT NUMBER 3(a) (FIG. 14) :-

a) RIGHT EYE :

The control eye, duration of the test 42 weeks. Ocular tension fluctuated around 15 mm.Hg.

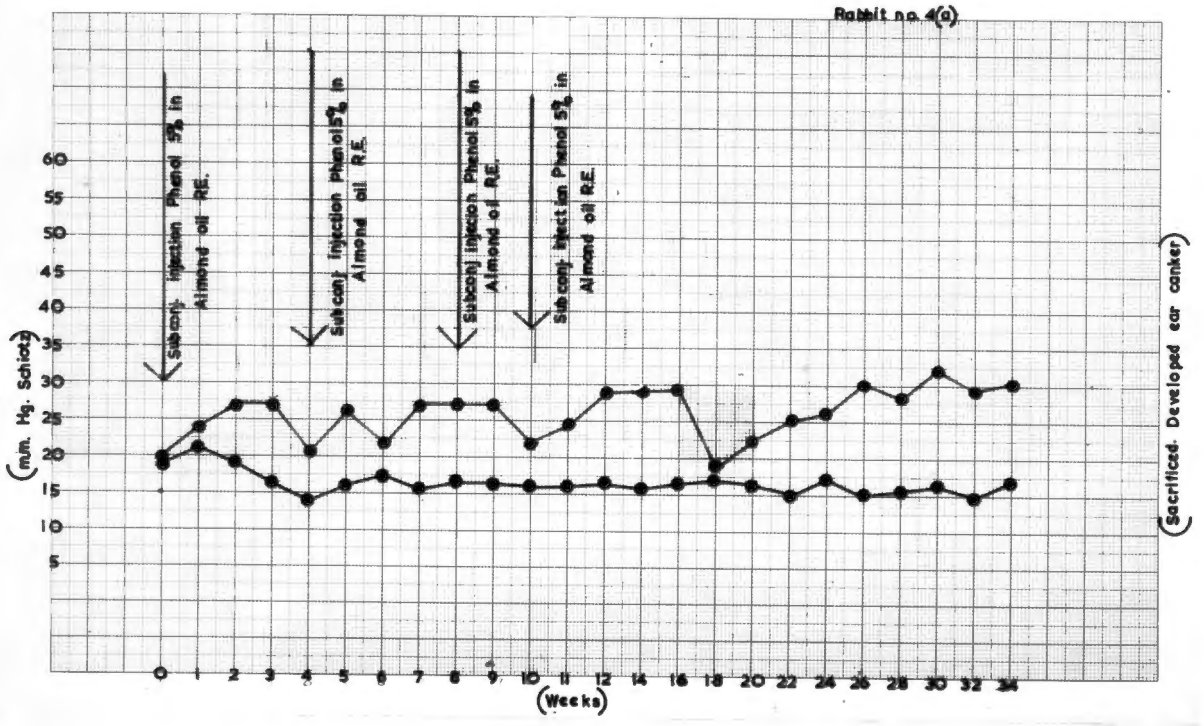
b) LEFT EYE :

Subconjunctival Phenol 5% in almond oil injected on 22 September 1961 and repeated at the 5th and again at the 8th week. The intraocular pressure remained consistently higher than the control eye, the base-line pressure before injection

/being

FIG. 15

Intraocular pressure in rabbit 4(a) plotted against time in weeks. Four subconjunctival injections given in the right eye are indicated. The left eye is the control.



being 16 mm.Hg. For the most part the intraocular pressure fluctuated between 25 and 30 mm.Hg.

RABBIT NUMBER 4(a) (FIG. 15) :-

The base-line intraocular pressure was 19 mm.Hg. in the left eye and 20 mm.Hg. in the right eye.

a) RIGHT EYE :

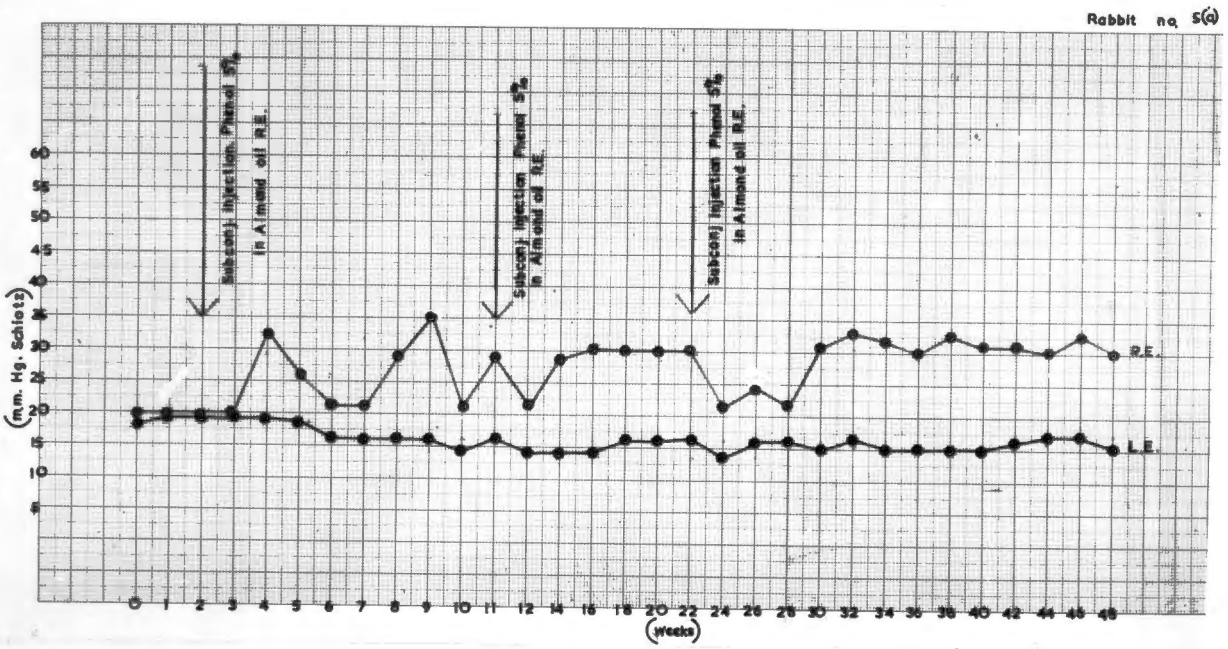
This was the injected eye, the first four-quadrant injection given on 22 September 1961 and repeated at the 4th, 8th and 10th week. Again the intraocular pressure remained consistently higher than in the left eye, fluctuating between 25 mm.Hg. and 30 mm.Hg. rarely exceeding this figure. At the 34th week the rabbit developed ear canker and had to be sacrificed.

b) LEFT EYE :

/This

FIG. 16

Intraocular pressure in rabbit No. 5(a) plotted against time in weeks. Three subconjunctival injections which were given in the right eye are indicated. The left eye is a control.



This was the control eye.

RABBIT NUMBER 5(a) (FIG. 16) :

The base-line intraocular pressure was 19 mm.Hg. in the left eye and 20 mm.Hg. in the right eye.

a) RIGHT EYE :

Phenol was injected subconjunctivally in four quadrants on the 25 September 1961 and repeated twice, at the 11th and 22nd week. Intraocular pressure varied between 20 mm.Hg. and 35 mm. Hg., remaining consistently higher than that in the left eye.

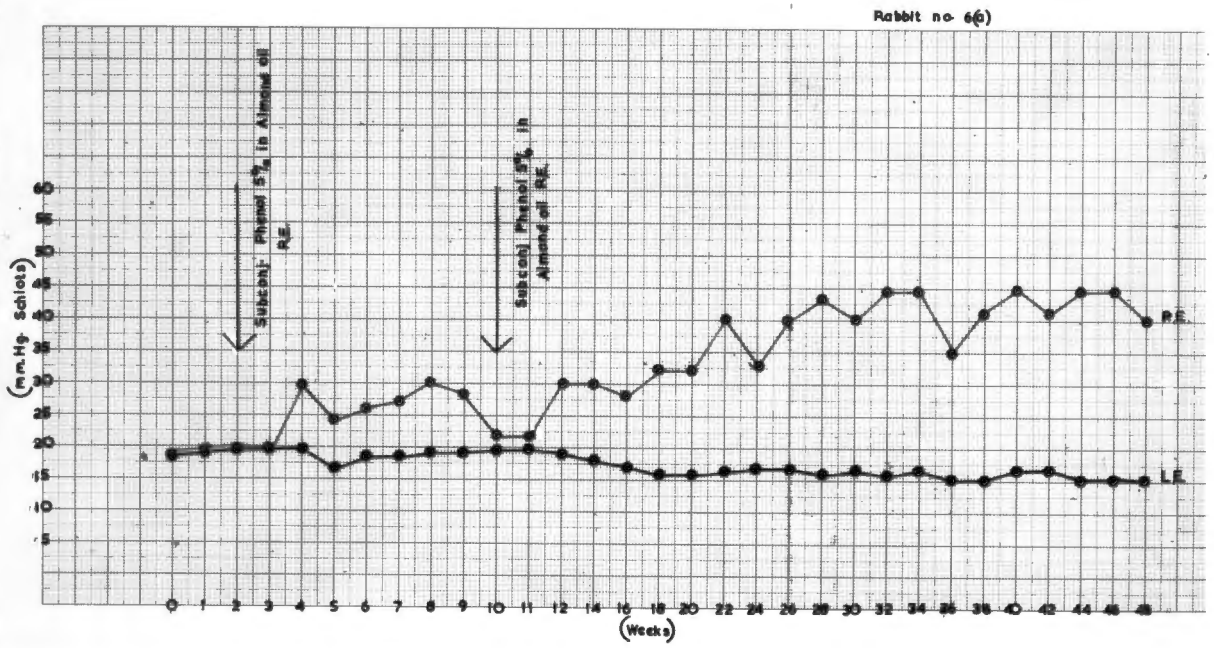
b) LEFT EYE :

The left eye was the control eye, the intraocular pressure remaining constant at 15 mm.Hg. The rabbit was sacrificed after 48 weeks.

/RABBIT

FIG. 17

Intraocular pressure in rabbit No. 6(a) plotted against time in weeks. Two subconjunctival injections given to the right eye are indicated. The left eye is a control.



RABBIT NUMBER 6(a) (FIG. 17) :-

a) RIGHT EYE :

Subconjunctival Phenol in almond oil was injected on the 25 September 1961 into four quadrants and repeated after 8 weeks. Intraocular pressure was 19 mm.Hg. and rose gradually, with a few fluctuations, to 45 mm.Hg. at the 46th week. The experiment was terminated at the 48th week.

b) LEFT EYE :

This was the control eye and had a base-line ocular tension of 19 mm.Hg. This fell gradually over the test period to 15 mm.Hg. at the 36th week, where it remained until the test was terminated at the 48th week.

/C) TONOGRAPHY

C) TONOGRAPHY :-

The test was carried out on all the rabbits in the second series.

The results, expressed as the "C" value, are summarized in Table I and the tonography curves are illustrated in figures 18 to 27.

The C values in all the treated eyes were well below the average normal value, and there was a significant difference between these values and the C values in the control eyes which were within normal limits.

These results indicate an increased resistance to aqueous outflow in the treated eyes.

SEE PAGE 94 FOR TABLE I

FIG. 18

Tonography recording of right eye of rabbit No. 2(a).

I.T. = Initial intraocular pressure at the start of the recording.

C = Facility of aqueous outflow.

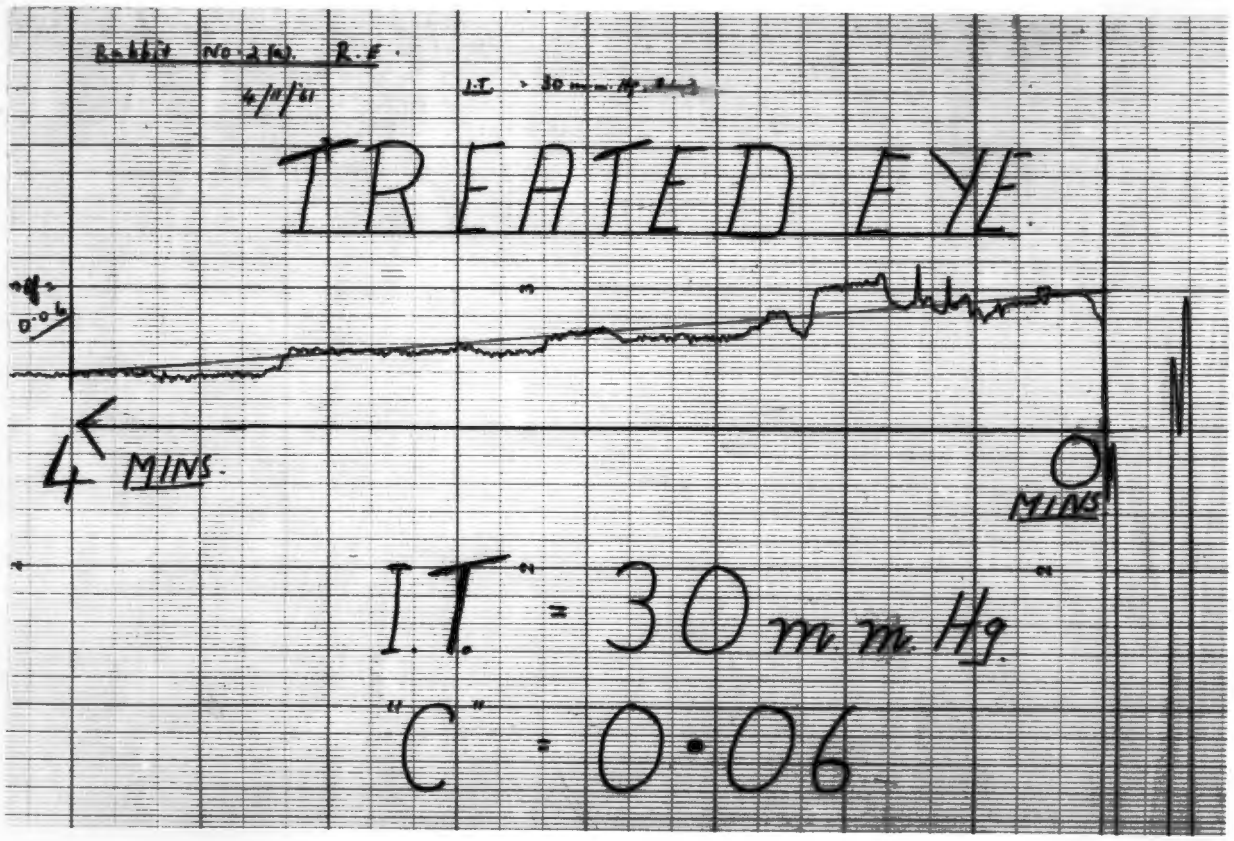


FIG. 19

Tonography recording of left eye of rabbit 2(a).

I.T. = Initial intraocular pressure at the start of the recording.

C = Facility of aqueous outflow.

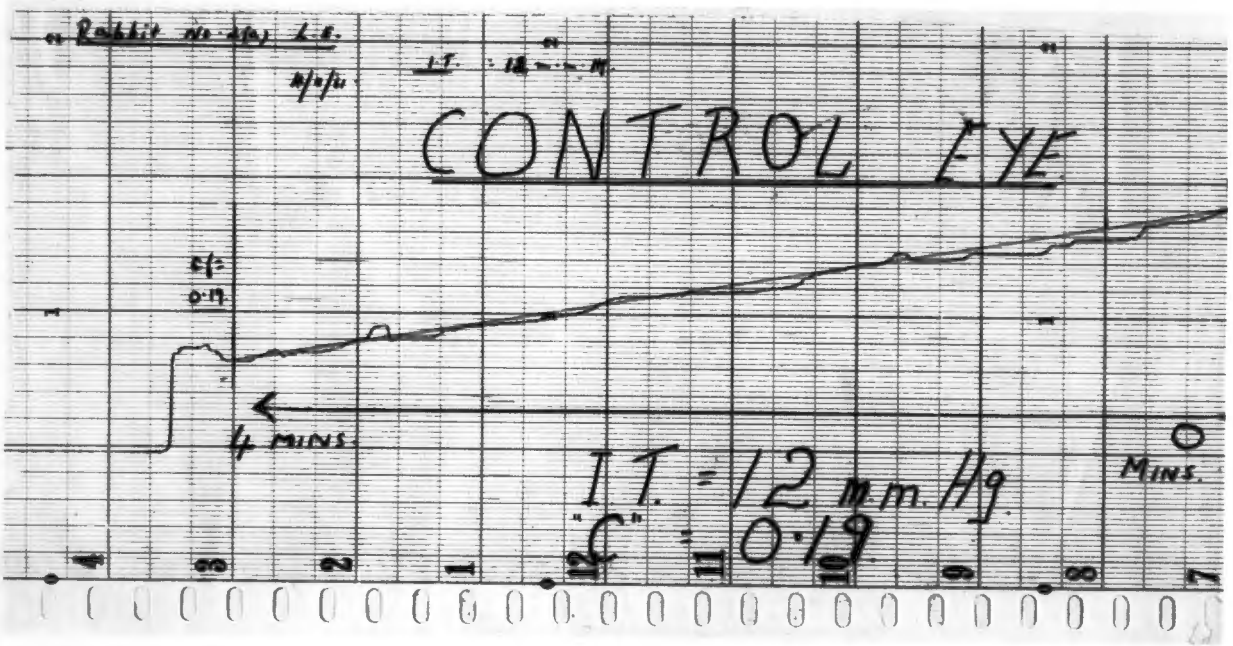


FIG. 20

Tonography recording of right eye of rabbit No. 3(a).

I.T. = Initial intraocular pressure at start of recording.

C = Facility of aqueous outflow.

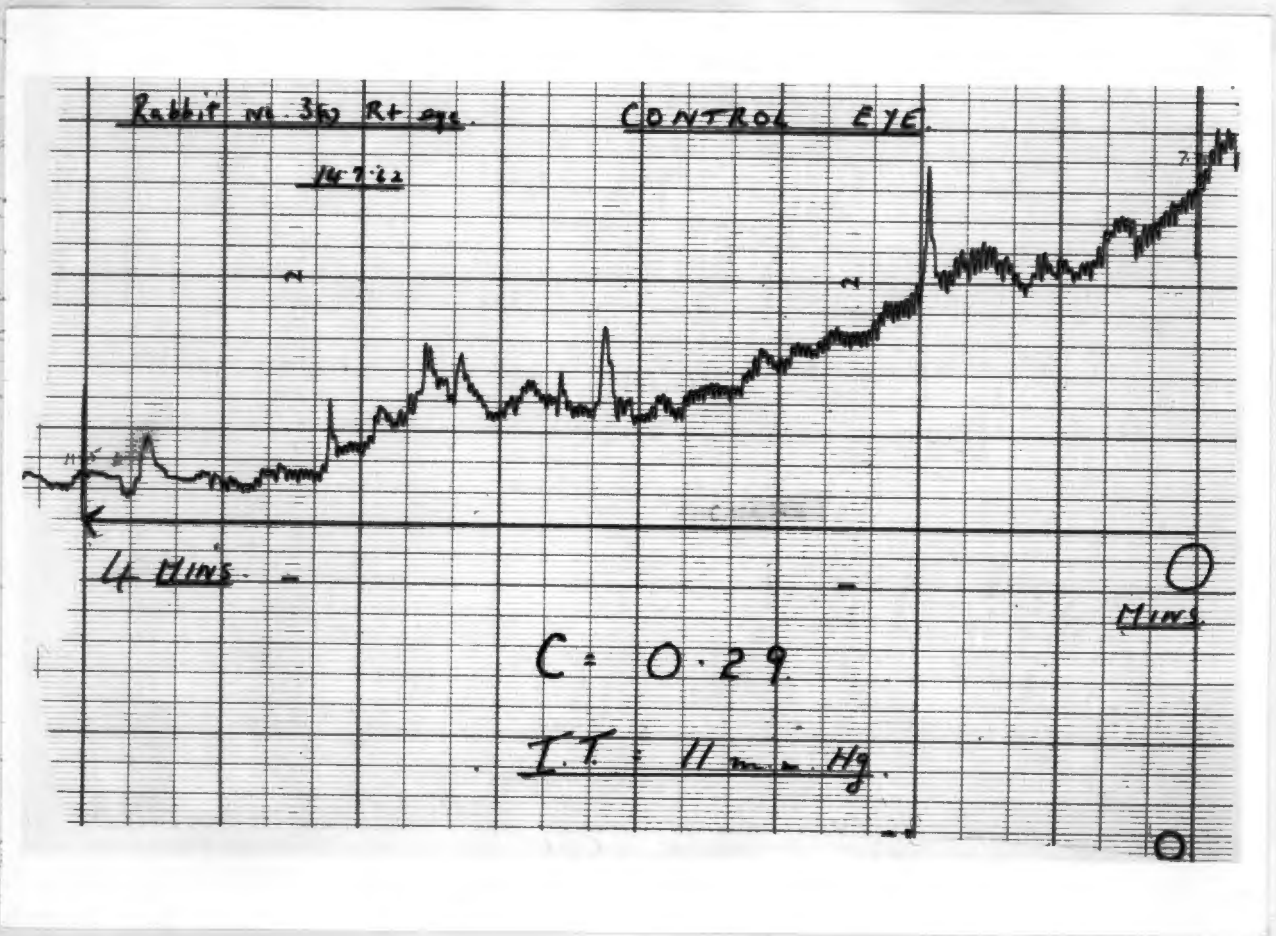


FIG. 21

Tonography tracing of left eye of rabbit 3 (a).

I.T. = Initial intraocular pressure at start of recording.

C = Facility of aqueous outflow.

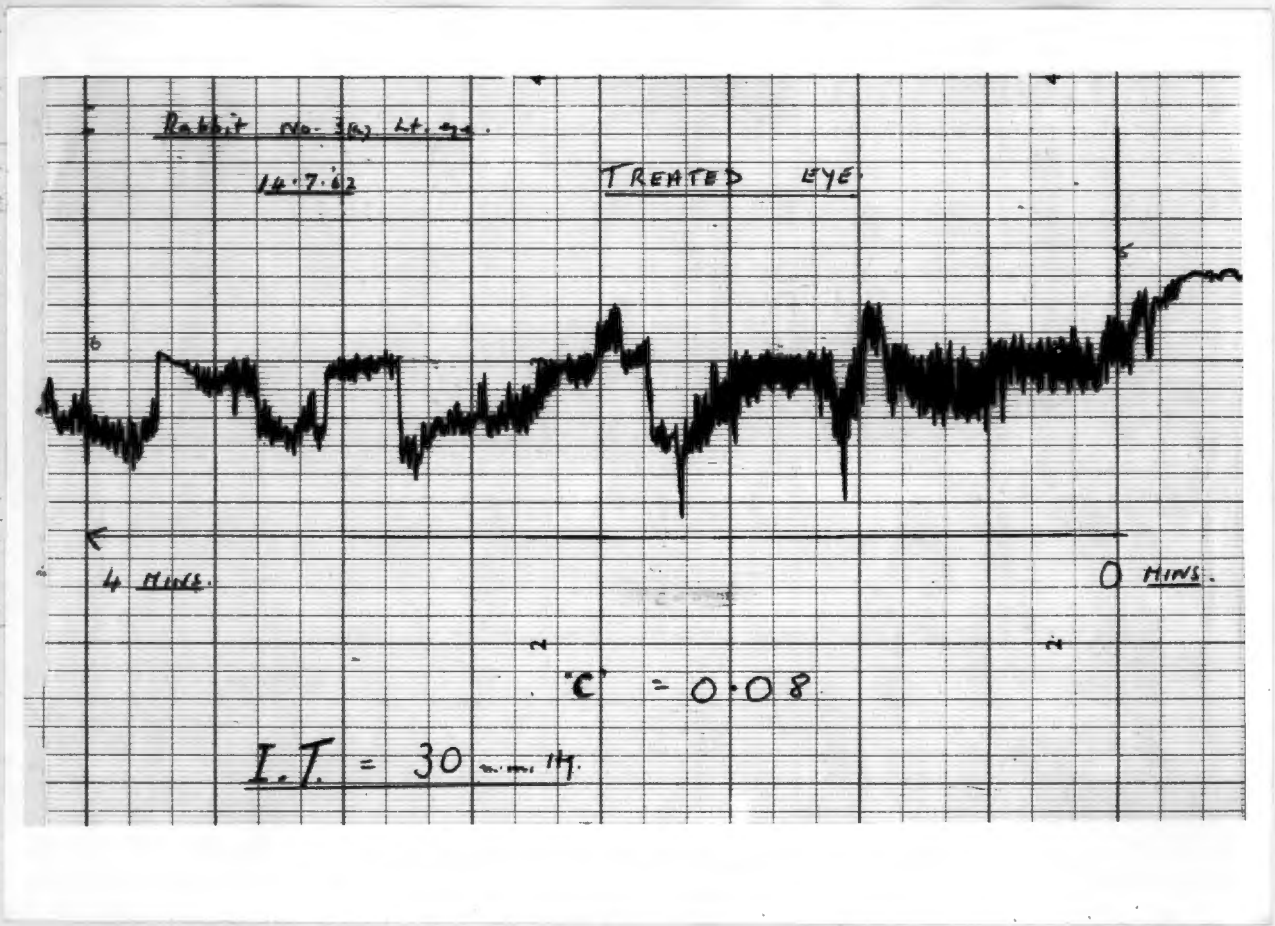


FIG. 22

Tonography tracing of right eye of rabbit 4 (a).

I.T. = Initial intraocular pressure at start of recording.

C = Facility of aqueous outflow.

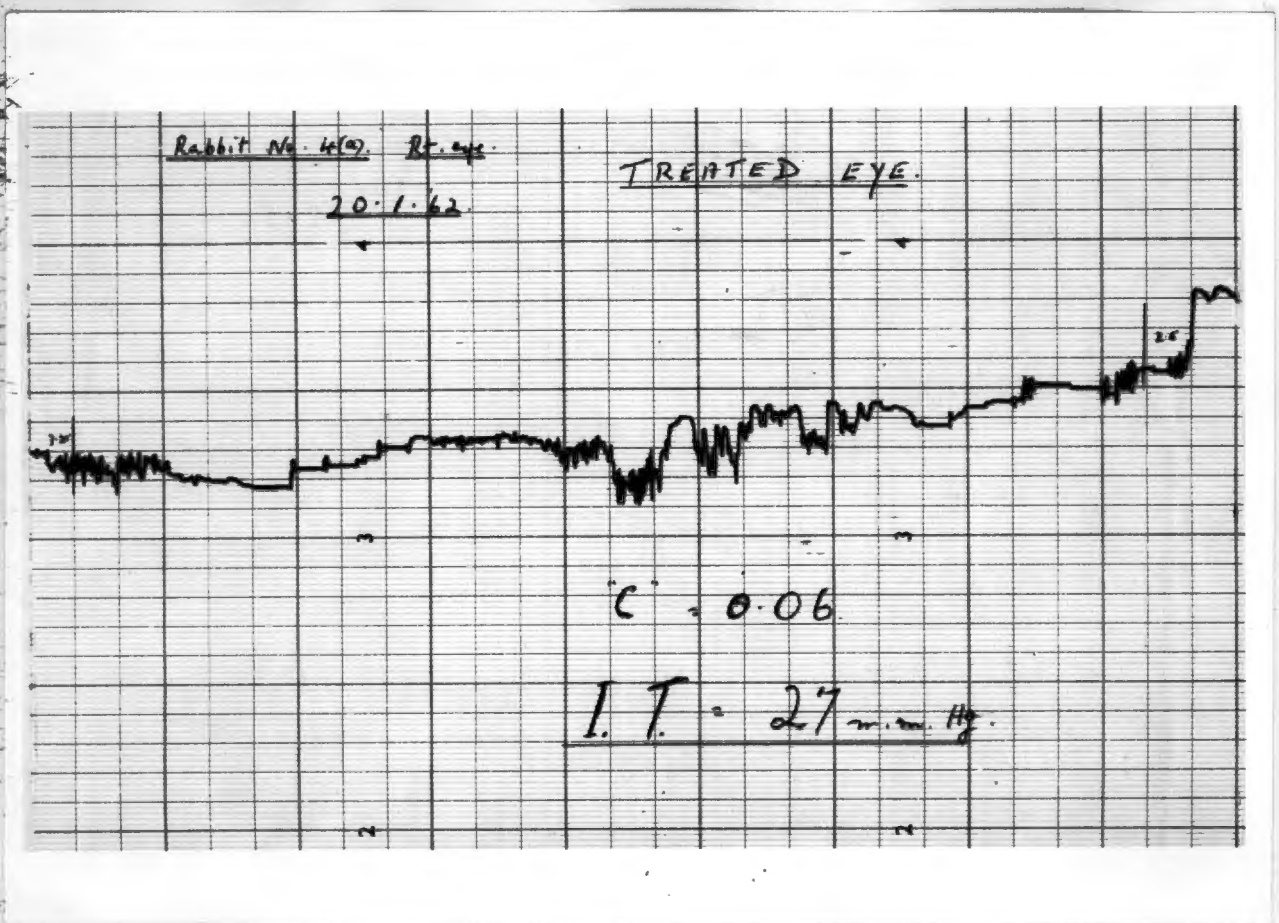


FIG. 23

Tonography record of left eye of rabbit 4(a).

I.T. = Initial intraocular pressure at start of recording.

C = Facility of aqueous outflow.

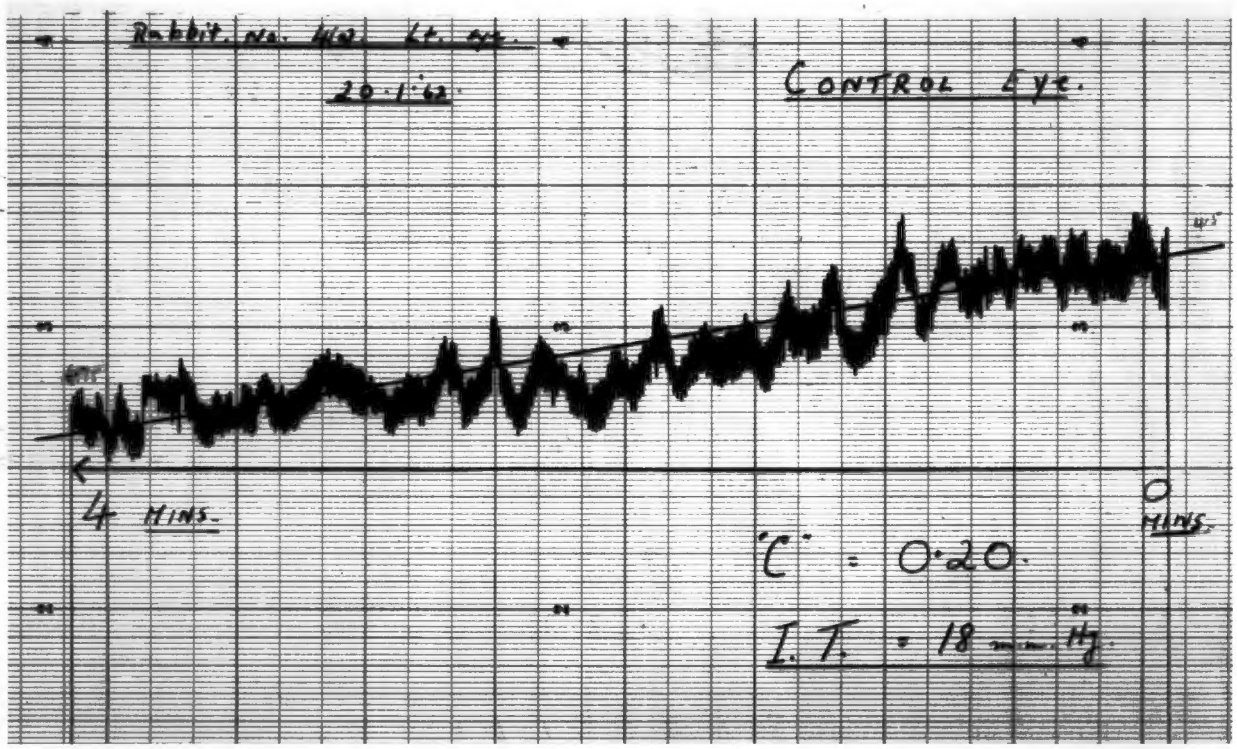


FIG. 24

Tonography record of right eye of rabbit 5 (a).

I.T. = Initial intraocular pressure at start of recording.

C = Facility of aqueous outflow.

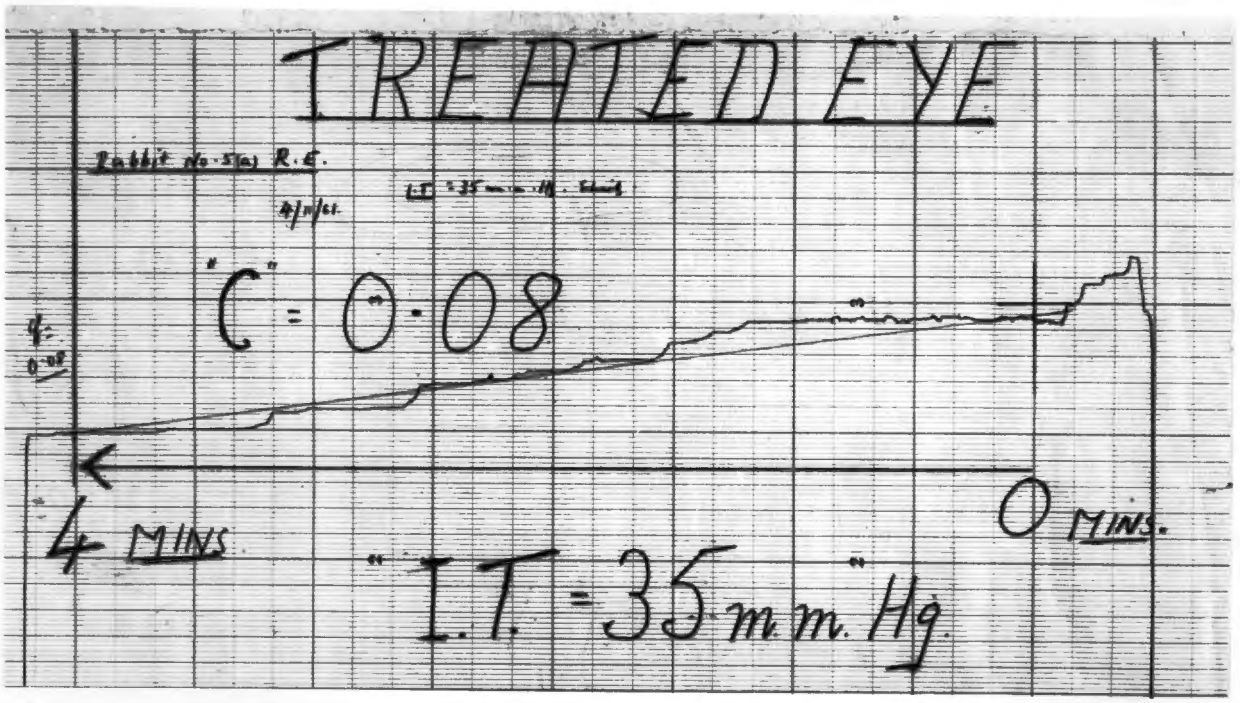


FIG. 25

Tonography tracing of left eye of rabbit No. 5(a).

I.T. = Initial intraocular pressure at start of recording.

C = Facility of aqueous outflow.

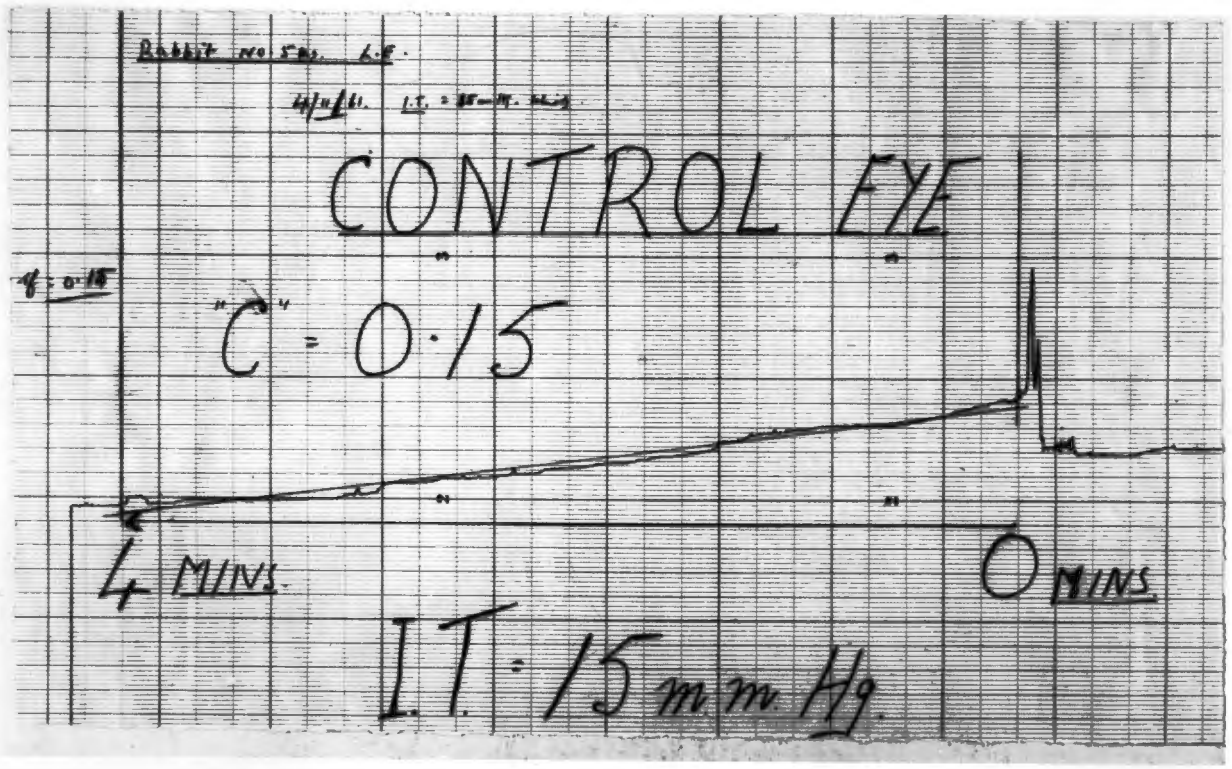


FIG. 26

Tonography tracing of right eye of rabbit No. 6(a).

I.T. = Initial intraocular pressure at start of recording.

C = Facility of aqueous outflow.

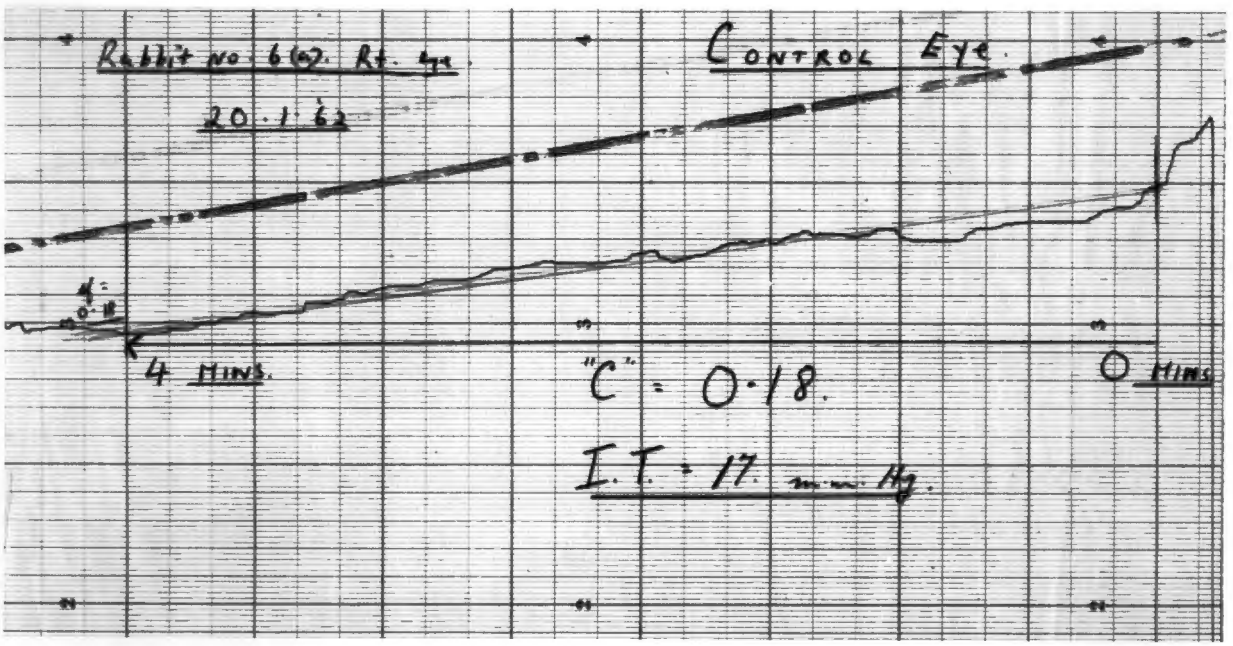


FIG. 27

Tonography tracing of left eye of rabbit No. 6(a).

I.T. = Intraocular pressure at start of recording.

C = Facility of aqueous outflow.

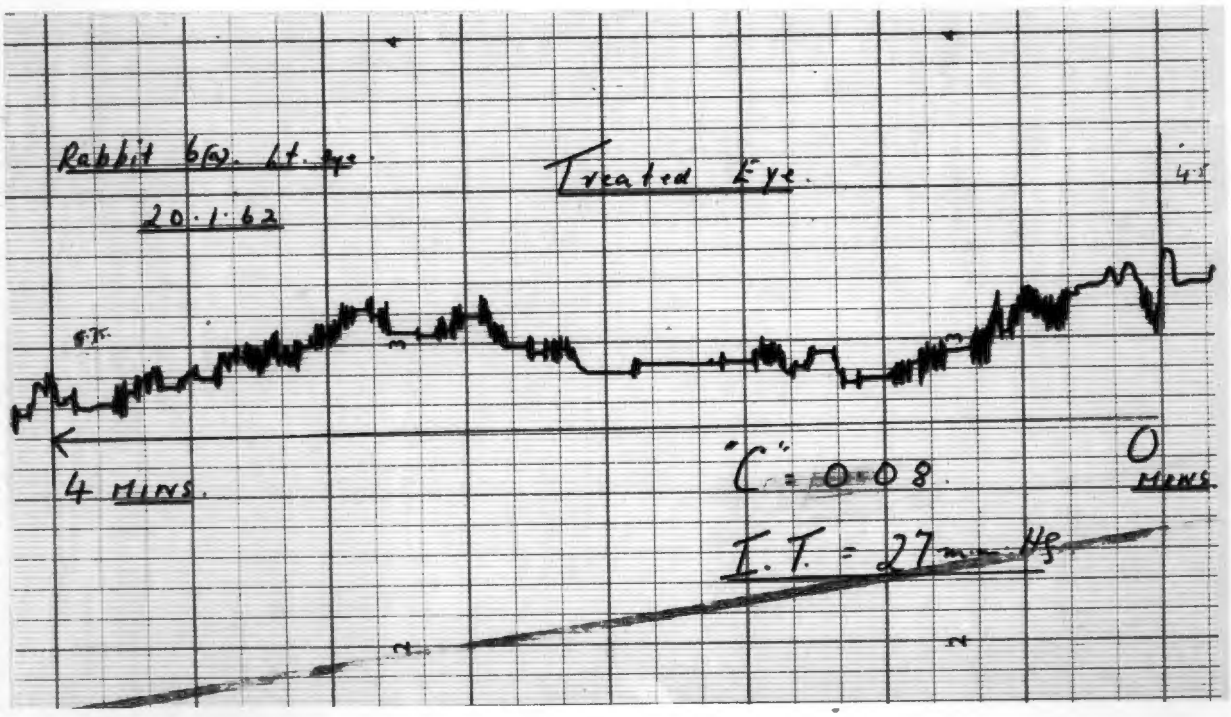


TABLE I

TONOGRAPHY

RABBIT	EYE	C	MEAN C	S.D.
2(a)Fig.18)	Right	0.06	a)Controls	a)Controls
Fig.19)	Left (c)	0.19		
3(a)Fig.20)	Right(c)	0.29	0.21	± 0.03
Fig.21)	Left	0.08		
4(a)Fig.22)	Right	0.06		
Fig.23)	Left (c)	0.20	b)Treated	b)Treated
5(a)Fig.24)	Right	0.08		
Fig.25)	Left (c)	0.15	0.07	± 0.02
6(a)Fig.26)	Right	0.08		
Fig.27)	Left (c)	0.18		

C = coefficient of resistance to outflow

(c) = control eye

S.D. = standard deviation

D) FLUORESCEIN APPEARANCE TIME TEST

The results of this test are summarized in Table II. Two results are recorded from each eye of the rabbits in

/the

the second series. Taking into account the inevitable crudeness of this test, these are remarkably constant. The treated eyes all showed prolonged fluorescein appearance time when compared to the controls (comparing treated and control eyes in the same animal), and this difference is obviously significant without having to resort to further statistical analysis. This observation showed that there had been a reduction in aqueous inflow in the treated eyes presumably as an attempt to compensate for the increased resistance to outflow. This had failed with the result that these eyes became glaucomatous. It was this compensatory process that dictated the slow rise of ocular tension, a situation analagous to the onset of chronic open angle glaucoma in man.

There is no absolute normal value for this test as it varies widely in

/different

different subjects, so that one can only compare an abnormal eye with the normal, preferably in the same animal.

SEE PAGE 97 FOR TABLE II

TABLE II

FLUORESCEIN APPEARANCE TIME TEST

RABBIT EYE		APPEARANCE TIME
2 (a)	Right	i) 2 min.20 sec. ii) 3 min.
	Left (c)	i) 1 min.45 sec. ii) 2 min. 5 sec.
3 (a)	Right(c)	i) 1 min.30 sec. ii) 1 min. 50 sec.
	Left	i) 3 min.30 sec. ii) 3 min. 10 sec.
4 (a)	Right	i) 2 min. 5 sec. ii) 2 min. 20 sec.
	Left (c)	i) 1 min.35 sec. ii) 1 min. 30 sec.
5 (a)	Right	i) 3 min.10 sec. ii) 3 min. 10 sec.
	Left (c)	i) 2 min. 5 sec. ii) 2 min. 20 sec.
6 (a)	Right	i) 3 min.30 sec. ii) 3 min.
	Left (c)	i) 2 min. ii) 1 min. 50 sec.

(c) = control eye.

DISCUSSION OF THE RESULTS :

These have been most encouraging. Glaucoma supervened in four out of five eyes treated with Phenol in almond oil in the first series, and in all treated eyes in the second series. A high level of intraocular pressure was reached in the first series and was maintained for an average period, in the four eyes, of 9.5 weeks, when the experiment was terminated.

The reaction to Pilocarpine and Diamox in two eyes in the first series and iris inclusion in the other two were in accord with what one would expect. This raises the possibility of using animal eyes prepared by this method to investigate the effectiveness of new preparations for the treatment of glaucoma, before applying these to man.

The level of ocular hypertension in the second series was not as striking as in the

/first

first. This may have been due to the different spacing in time of the injections, or to the use of a different breed of animal. Nevertheless, consistently raised pressures often reaching over 30 mm.Hg. were maintained in the treated eyes for an average period of 40 weeks, when the experiment was terminated. During the same time, aqueous outflow resistance and aqueous inflow time were increased in these eyes.

I attempted to produce an outflow glaucoma by obliterating the aqueous outflow channels. That I succeeded in doing this is shown by the tonography results which recorded low C values in the treated eyes, and indirectly by the delayed fluorescein appearance time suggesting a reduced aqueous inflow and turnover in the glaucomatous eyes.

No gross ocular damage could be detected by either macroscopic or microscopic examination of the treated eyes. (See Section VIII).

/The

The intraocular pressure in the control eyes remained constant. The average tension readings compared well with those from other series. This indicated that the methods used for measuring intraocular pressure and aqueous outflow are valid. It would be appropriate at this stage to examine in more detail the validity of these methods.

SECTION VII

VALIDITY OF METHODS USED FOR MEASURING INTRAOCULAR PRESSURE AND AQUEOUS OUTFLOW

These methods have already been described. I have earlier in this paper submitted that they can be used with a high degree of clinical accuracy. I now propose to show that the results obtained in this experiment are valid by comparing the measurements in my control eyes with measurements obtained from normal rabbit eyes by other investigators. There should be close agreement and in fact this is what one finds. Secondly, the measurements obtained from the control eyes show very little scatter around the mean value when expressed by the standard deviation from the mean.

a) INTRAOCULAR PRESSURE :

In Table III, the mean ocular tension (Po) in the control eyes in my series is compared with the figures

/obtained

obtained by Becker and Constant (1956) when they measured the intraocular pressure in normal rabbit eyes in vivo, using a standardized, weighted Schiötz tonometer. There is good agreement.

TABLE III

Series	Mean Ocular Tension (Po)	Standard Deviation
i) Present series	17.8 mm.Hg.	± 1.9
ii) Becker and Constant series	18.0 mm.Hg.	± 3.7

The standard deviations are not large, striking evidence for the accuracy of the Schiötz tonometer, used under these conditions, for measuring intraocular pressure in rabbits.

b) TONOGRAPHY :

Table IV compares the mean C value

/in

in my series of control rabbit eyes with the mean C values obtained by Becker and Constant (1956) and by Kornblüth and Linnér (1955) and Levene and Bloomgarden (1961) when measuring facility of outflow in normal rabbit eyes, using a similar technique as in the present study. The latter authors also showed that there was no significant difference in results between right and left eyes; the right eyes were measured first.

T A B L E IV

Series	Mean C Value	Standard Deviation
i) Present series (1962)	0.21	± 0.03
ii) Kornblüth & Linnér (1955)	0.30	± 0.04
iii) Becker & Constant (1956)	0.34	± 0.10
iv) Levene & Bloomgarden (1961)	0.26	± 0.06

/The

The standard deviation about the mean is small, underlying the consistency of the measurements. The C value in the present series is lower than in the other three, but well within the range of normal, which is 0.14 to 0.60. This emphasises very clearly that tonography is not a scientifically accurate measurement of resistance to outflow but is nevertheless a useful clinical test as long as it is accepted that there is a large range for normal values. The value obtained depends to a great extent on environmental conditions pertaining at the time of the test. In the four series quoted in Table IV, although the test was done by different investigators under vastly differing conditions, the mean values all lie within the normal range and the standard deviations for each series are small.

In my opinion Table III provides sufficient evidence that the methods employed in

/this

this experiment have provided valid results. If one accepts that the results are valid, then the technique that I have described is a useful method of producing chronic outflow glaucoma in animals which closely resembles primary glaucoma in man. The possibilities of studying the effects of various drugs or surgical procedures on these eyes is immediately obvious. One hopes that these experiments will emphasize the importance of the episclera and in particular the episcleral vessels in the regulation of aqueous humour flow and dynamics and its possible role in the aetiology of primary glaucoma.

S E C T I O N VIII

PATHOGENESIS : HISTOLOGICAL STUDIES ON GLAUCOMATOUS AND CONTROL RABBIT EYES

Seven glaucomatous and five control eyes were obtained for histological study by killing six of the rabbits used in this experiment (numbers 3, 2(a), 3(a), 4(a), 5(a) and 6(a)). Another rabbit that failed to develop glaucoma (number 4) was also killed and its eyes removed for histological study. Two rabbits that had iris inclusions performed on their eyes (numbers 5 and 6) were not included in this study because it was considered that the histology might be significantly altered by the operation.

The eyes were embedded in Celloidin, sectioned and stained with haematoxylin and eosin and van Giesen's in the usual way. Photomicrographs of sections stained with haematoxylin and eosin are presented in Figures 28 to 41.

/I

FIG. 28

Section of conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the right eye of rabbit 3 (glaucomatous eye).
H & E stain. x 100.

FIG. 29

Section of conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the left eye of rabbit 3 (glaucomatous eye).
H & E stain. x 100.

FIG. 30

Section through conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the right eye of rabbit 4 (failed to develop glaucoma).
H & E stain. x 100.

FIG. 31

Section through conjunctiva, episclera and sclera at the angle of the anterior chamber of the left eye of rabbit 4 (control eye). H & E stain. x 100.

FIG. 32

Section through conjunctiva, episclera and sclera at the angle of the anterior chamber of the right eye of rabbit 2(a) (control eye). H & E stain. x 100.

FIG. 33

Section through conjunctiva, episclera and sclera at the angle of the anterior chamber of the left eye of rabbit 2(a) (glaucomatous eye).

H & E stain. x 100.

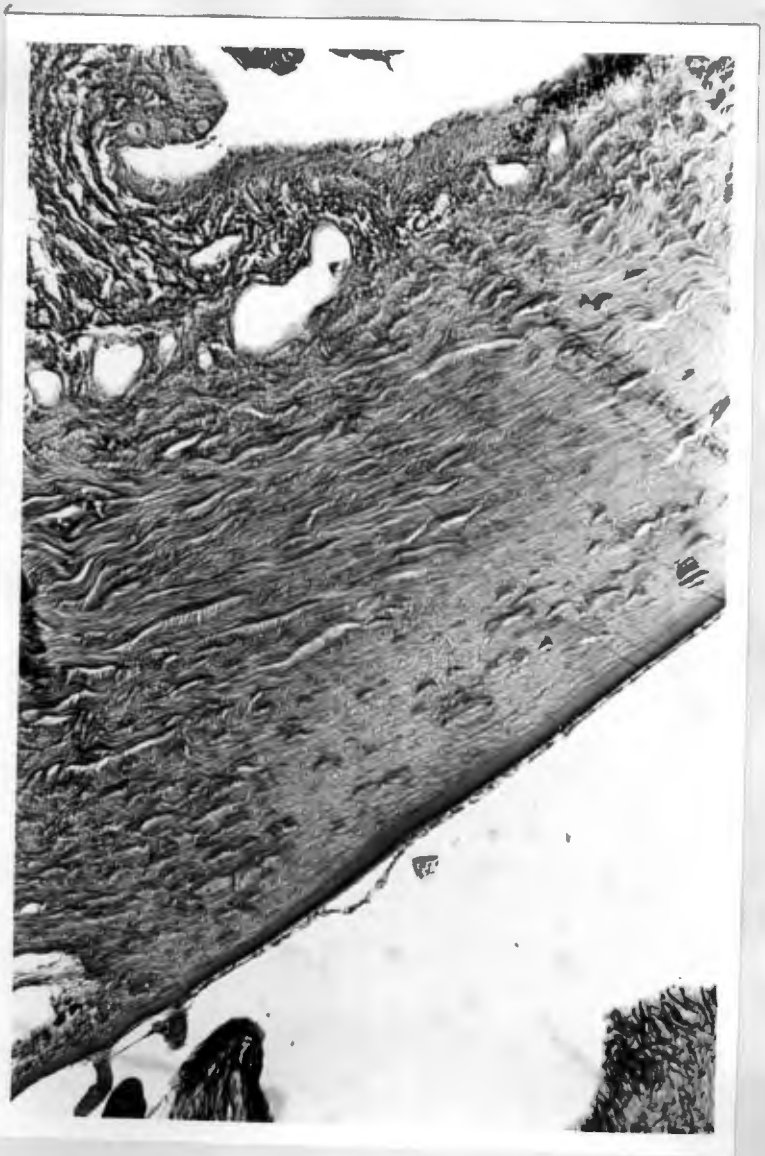


FIG. 34

Section through conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the right eye of rabbit 3(a) (control eye).
H & E stain. x 100.

FIG. 35

Section through conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the left eye of rabbit 3(a) (glaucomatous eye).
H & E stain. x 100.

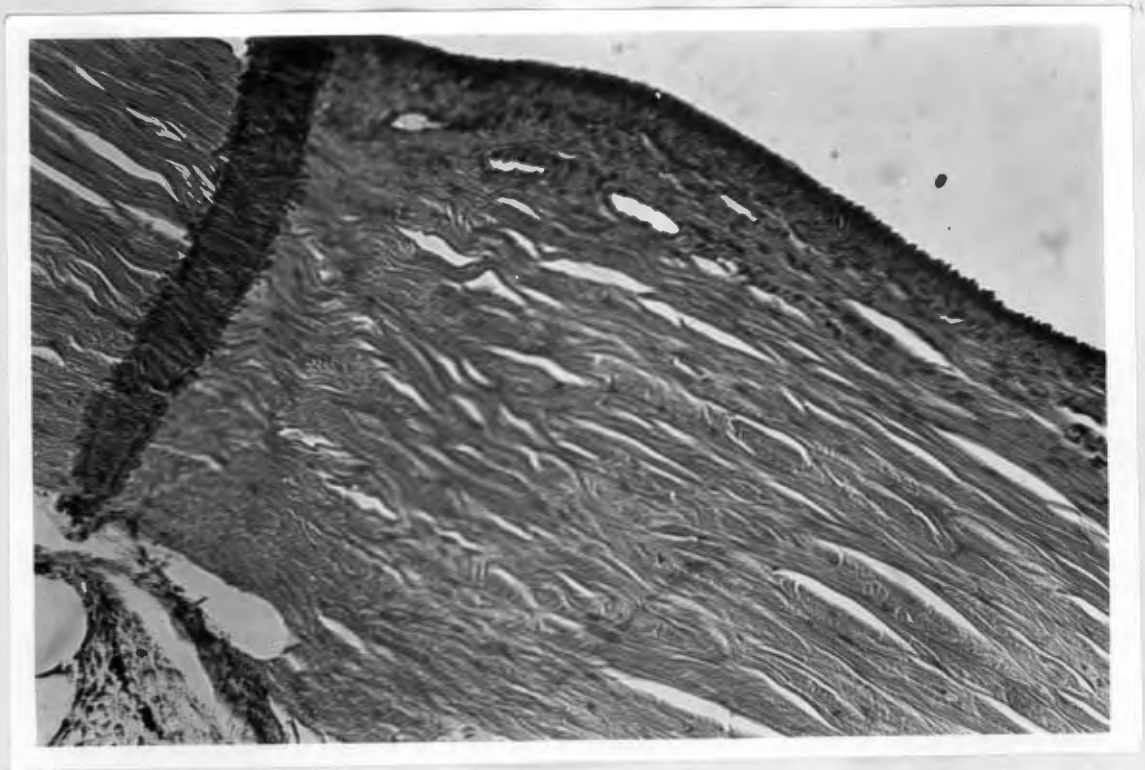


FIG. 36

Section through conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the right eye of rabbit 4(a) (glaucomatous eye).
H & E stain. x 100.

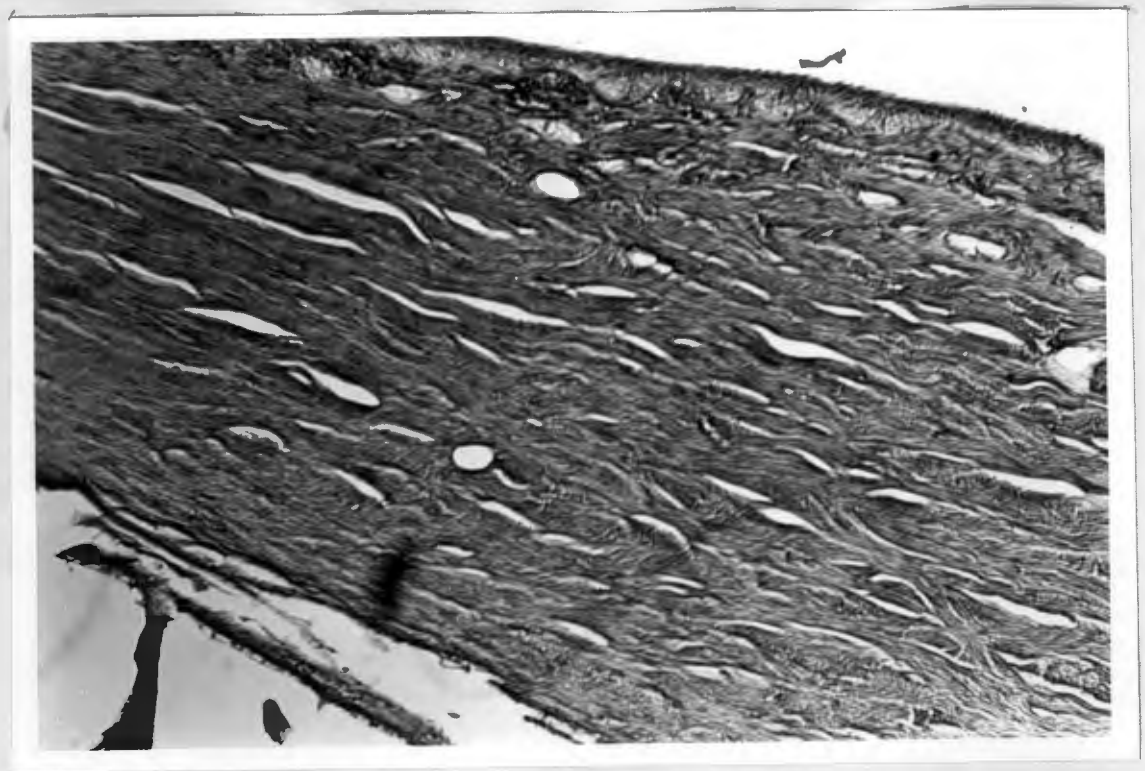


FIG. 37

Section through conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the left eye of rabbit 4(a) (control eye).
H & E stain. x 100.

FIG. 38

Section through conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the right eye of rabbit 5(a) (glaucomatous eye).
H & E stain. x 100.

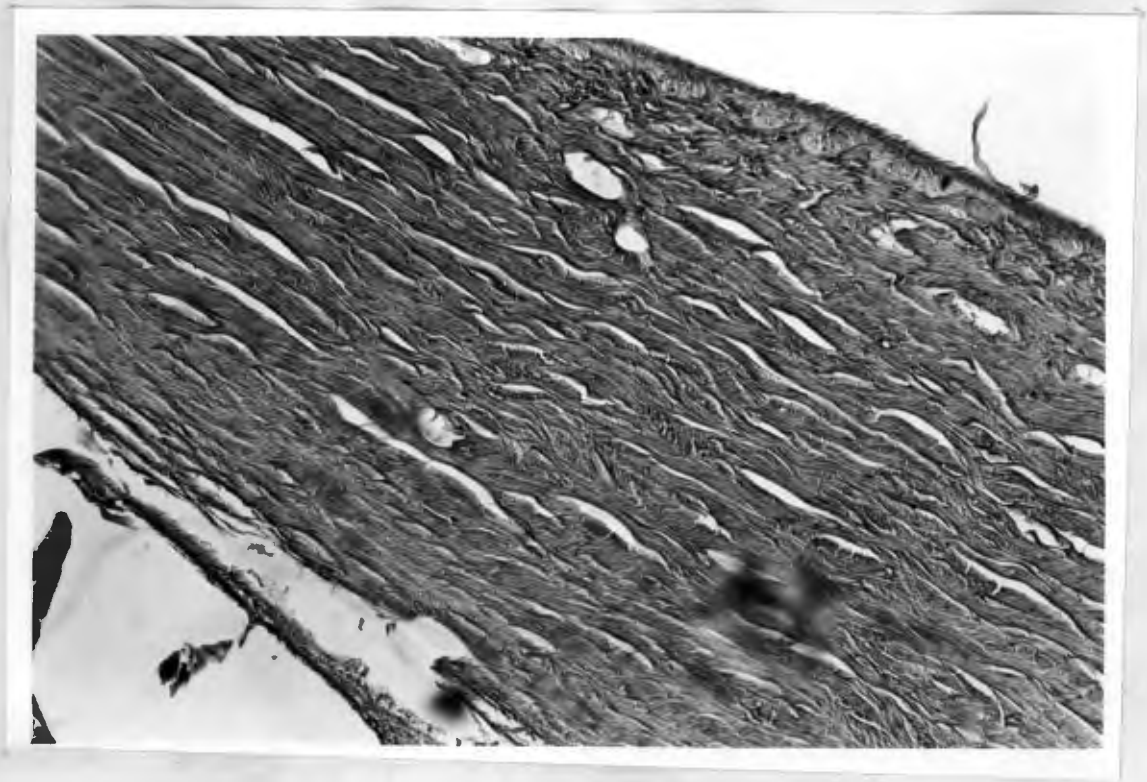


FIG. 39

Section through conjunctiva, episclera and sclera in the region of the angle of the anterior chamber of the left eye of rabbit 5(a) (control eye).
H & E stain. x 100.

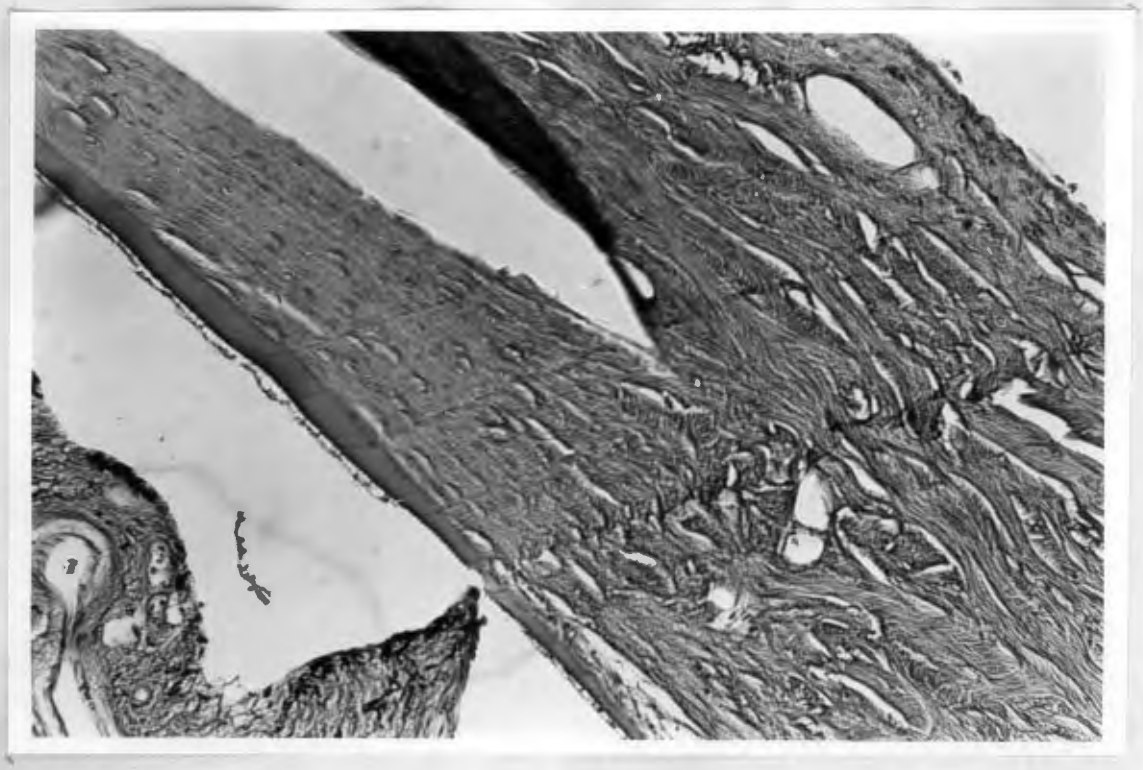


FIG. 40

Section of conjunctiva, episclera and sclera at the angle of the anterior chamber of the right eyeof rabbit 6(a) (glaucomatous eye). H & E stain. x 100.

FIG. 41

Section of conjunctiva, episclera and sclera at the angle of the anterior chamber of the left eye of rabbit 6(a) (control eye).
H & E stain. x 100.

I carefully studied all the sections, examining many sections at different levels from each eye but could not detect any significant difference between glaucomatous and control eyes. Sections from the eyes of rabbit 4 also appeared to be normal.

Because it was important to be absolutely certain that I had not missed any pathological changes, I consulted Dr. Robb-Smith, Nuffield Reader in Pathology at the University of Oxford and also Dr. Michael J. Hogan, School of Medicine, Department of Ophthalmology, University of California, San Francisco, U.S.A. who confirmed my interpretation of these sections (see page 120). Dr. Robb-Smith went as far as to suggest that should the pathogenesis of glaucoma in these eyes be diffuse episcleral fibrosis and obliteration of the smallest episcleral capillary plexus's, this could escape detection by ordinary histological methods. I also consulted Professor J.G. Thompson,

/Professor

Professor of Pathology at the University of Cape Town, who agreed with Dr. Robb-Smith's opinion.

The next step was a study of the outflow channels from the anterior chamber in glaucomatous and control rabbit eyes, paying special attention to the scleral and episcleral outflow. I was able to do this with facilities provided by Professor A. Kipps and Dr. Golda Selzer in the virus research unit of the University of Cape Town and I am happy to have this opportunity of acknowledging my debt to them.

Eight rabbits with normal eyes (sixteen eyes) were selected from the stock at the University of Cape Town Medical School of the same species as had been used in my initial experiment. Only rabbits with an average scleral rigidity were used and this was tested by the method described on page 60. The intraocular pressure was checked in each of the sixteen eyes on three separate occasions at intervals of

/approximately

approximately four days and found to be less than 18.5 millimetres of mercury in each eye.

Ten of these rabbit eyes were injected subconjunctivally with phenol in almond oil according to the method described on pages 36 to 39. Of the ten eyes so treated, eight developed an intraocular pressure of between 30 and 40 millimetres of mercury (Table V) after a period of six weeks from the first subconjunctival injection, and were considered to have developed glaucoma. They were now ready for the next stage of the experiment.

The six rabbit eyes which were not subjected to subconjunctival injections were used as control eyes. In these eyes the intraocular pressure varied between 16 to 18 millimetres of mercury .

TABLE V PAGE 112

FIG. 42

Photomicrograph of a section through the sclera and episclera at the anterior chamber angle of a glaucomatous rabbit eye enucleated 2 hours after an anterior chamber injection of 0.5 milliliter of indian ink. Drainage of indian ink through the sclera had not yet commenced.
H & E stain. x 320.

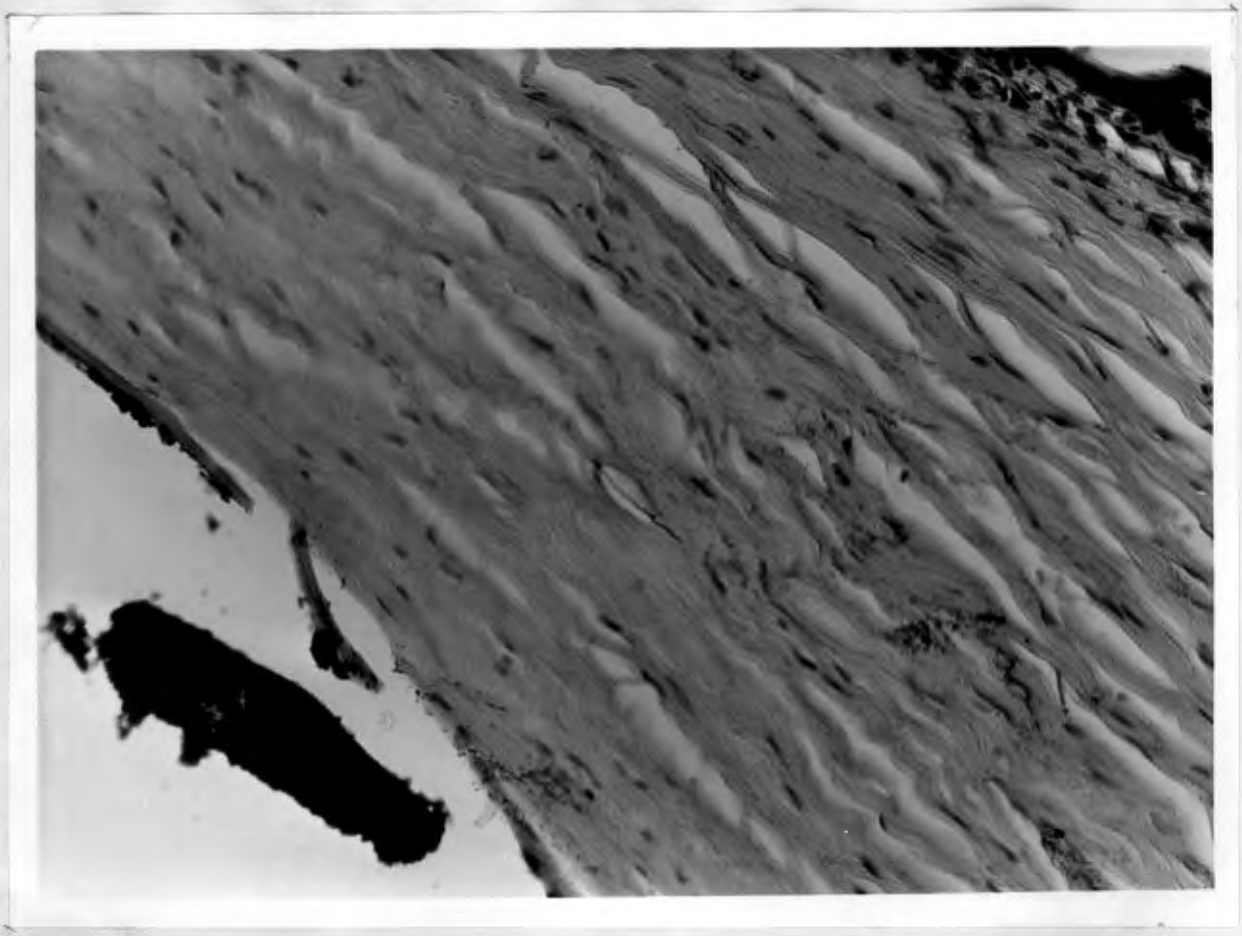


FIG. 43

Photomicrograph of a section through the sclera and episclera at the anterior chamber angle of a control rabbit eye removed 2 hours after an injection of 0.5 milliliter of indian ink into the anterior chamber. There was indian ink in the anterior chamber but no sign of drainage through the sclera. H & E stain. x 320.

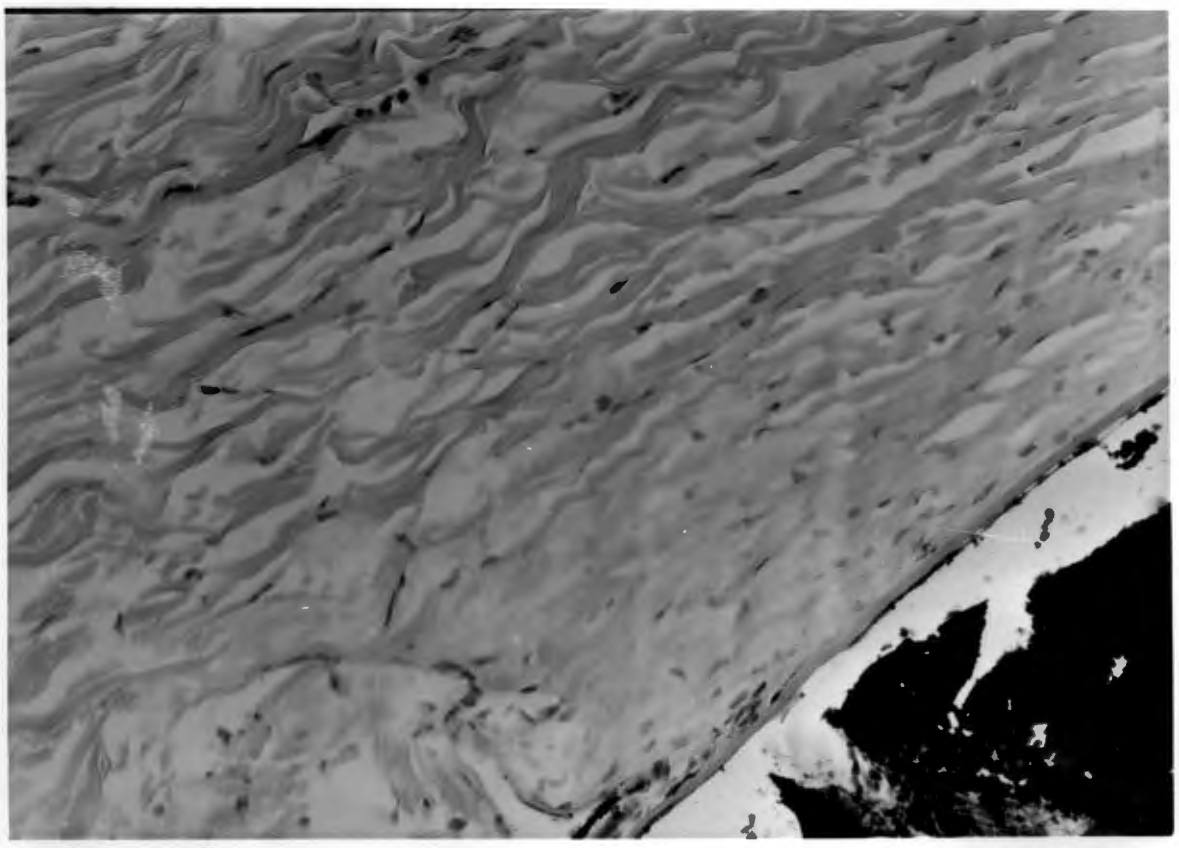


FIG. 44

Photomicrograph of a section through the sclera and episclera at the angle of the anterior chamber from a glaucomatous rabbit eye removed 24 hours after an injection of 0.5 milliliter of indian ink into the anterior chamber. Indian ink was present in the superficial scleral layers but had not reached the episclera. This was the only section from a glaucomatous eye in which indian ink had drained as far as the superficial scleral layers.
H & E stain. x 320.

SEE OVERLEAF

FIG. 44

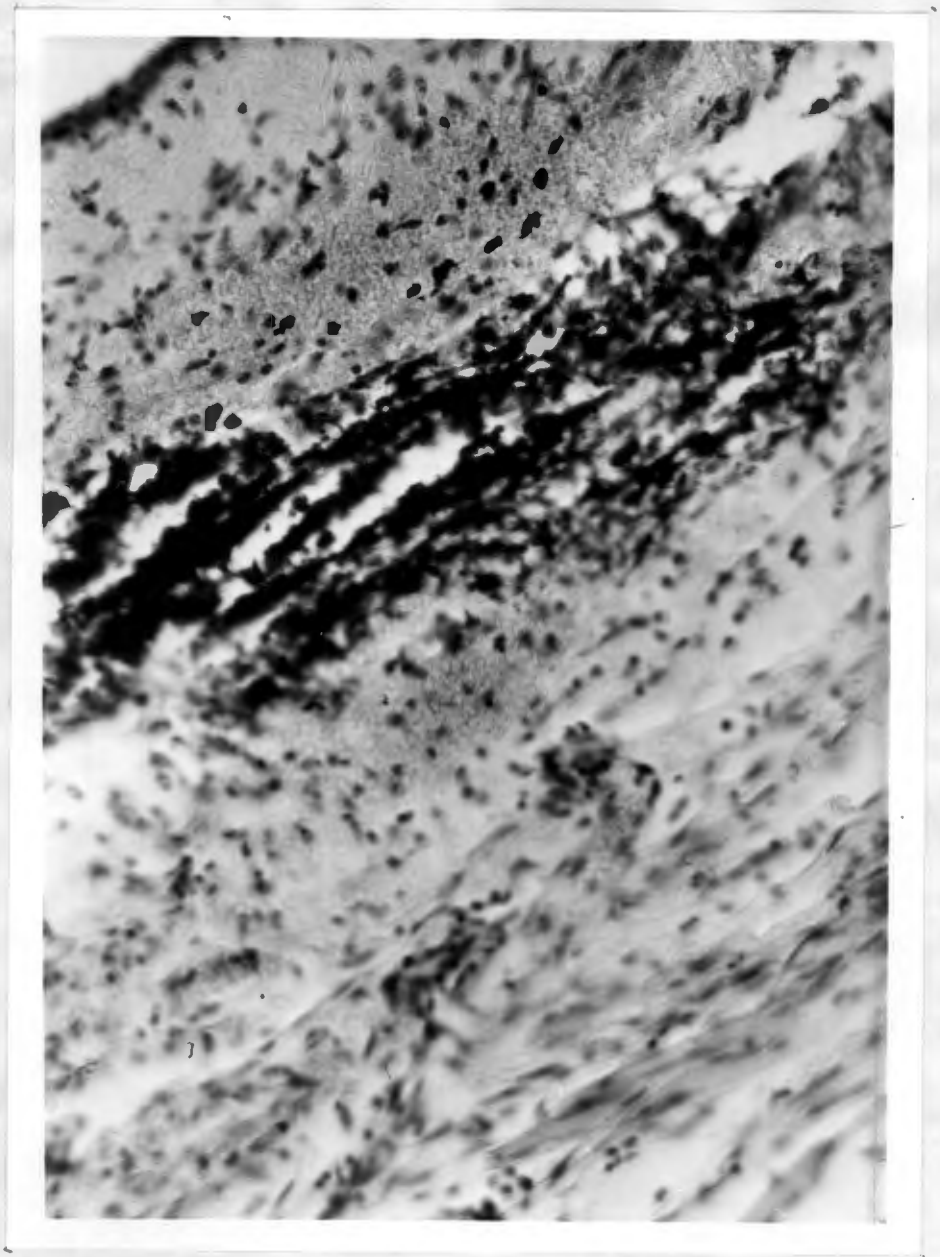


FIG. 45

Photomicrograph of a section through sclera and episclera from a glaucomatous rabbit eye near the angle of the anterior chamber. The eye was removed 24 hours after an injection of 0.5 milliliter of indian ink into the anterior chamber. Indian ink had drained into the deeper scleral plexus but no farther. This was the appearance in most of the sections from eyes with glaucoma. H & E stain. x164.

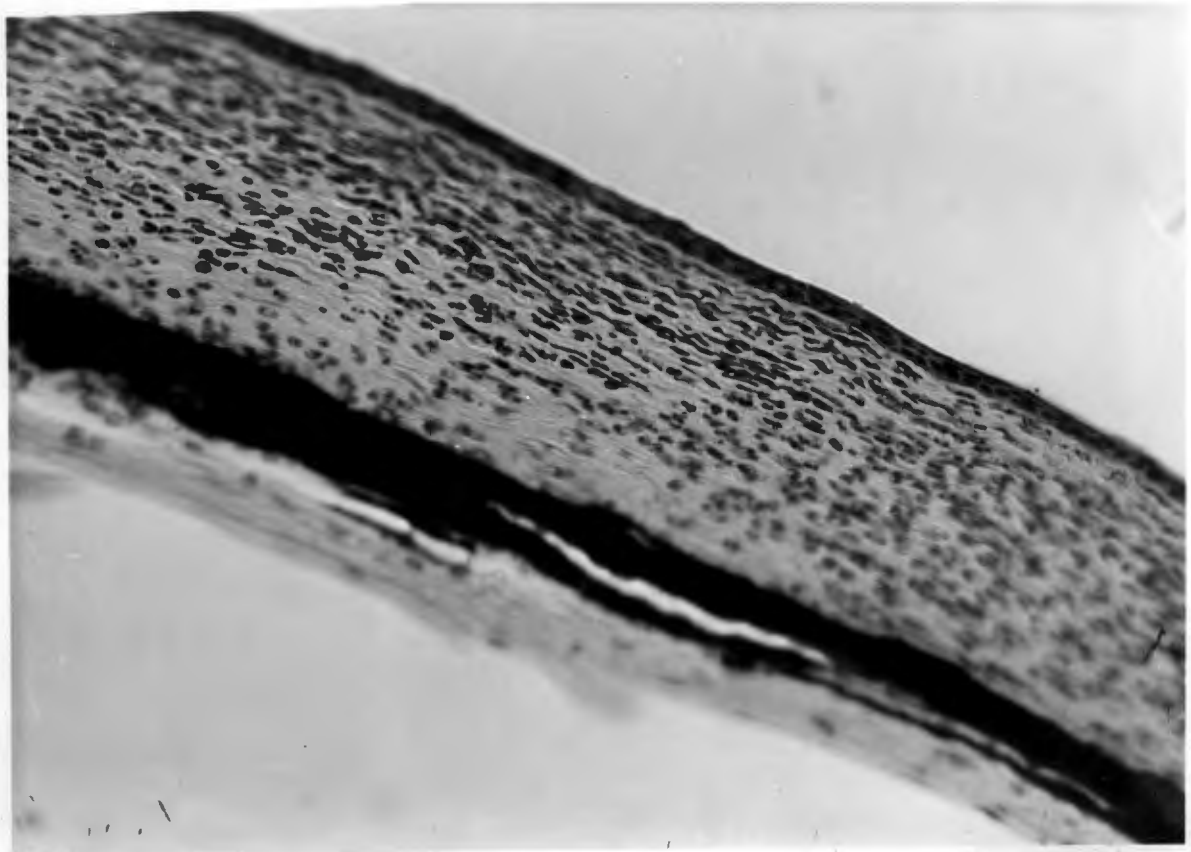


FIG. 46

Photomicrograph of a section through sclera and episclera from a control rabbit eye at the angle of the anterior chamber. The eye was enucleated 24 hours after an injection of 0.5 milliliters of indian ink into the anterior chamber. Indian ink had drained into the episclera. Unfortunately the episclera became distorted during preparation of this slide but indian ink can clearly be seen within the episcleral tissue. Sections from the other control eyes similarly demonstrated the drainage of indian ink into the episclera.

H & E stain. x 320.

SEE OVERLEAF

FIG. 46

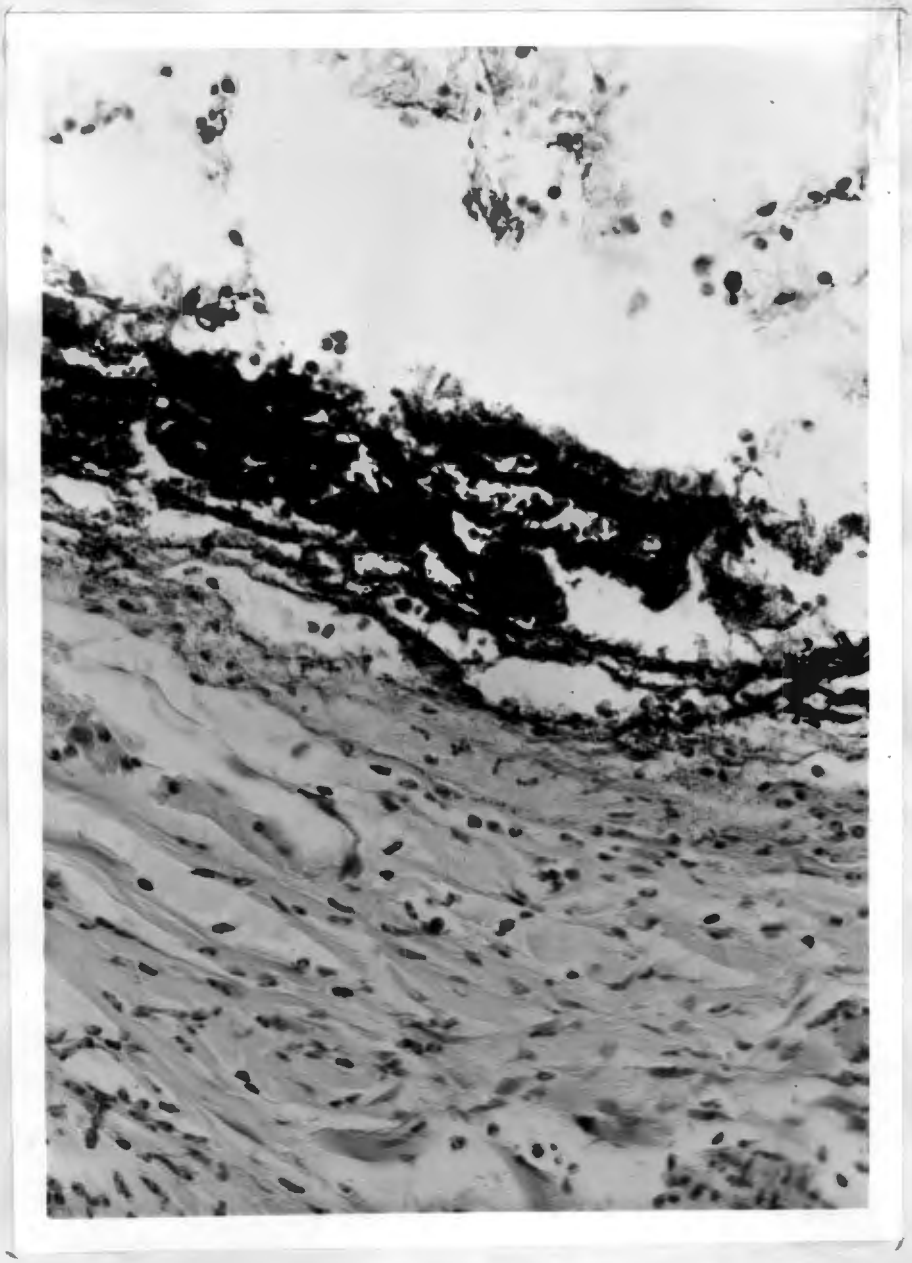


TABLE V

INTRAOCULAR PRESSURE IN EIGHT RABBIT EYES WITH GLAUCOMA WHEN INJECTED WITH INDIAN INK.

EYE	INTRAOCULAR PRESSURE	EYE ENUCLEATED AT
1	34.5 millimetres mercury	2 hrs.
2	32.0 millimetres mercury	2 hrs.
3	31.6 millimetres mercury	2 hrs.
4	42 millimetres mercury	24 hrs.
5	34.5 millimetres mercury	24 hrs.
6	33 millimetres mercury	24 hrs.
7	35 millimetres mercury	24 hrs.
8	37.8 millimetres mercury	24 hrs.

In the six control eyes the intraocular pressure fluctuated between 16 millimetres mercury and 18 millimetres mercury.

/Undiluted

Undiluted indian ink (0.5 milliliter) was injected into the anterior chamber of each of these 14 rabbit eyes (8 glaucomatous eyes and 6 control).

The animal was immobilized and the eye anaesthetized in the same way as described for tonometry (page 34). Indian ink was delivered into the anterior chamber from a standard 2 cubic centimetre syringe with a number 18 needle through the cornea at the limbus.

Three glaucomatous eyes and three control eyes were removed after two hours for histological study and the remaining five glaucomatous and three control eyes after 24 hours. These eyes were halved, embedded in paraffin, sectioned and stained with haematoxylin and eosin in the usual way. Sections were taken at three different levels from each half of each eye.

Careful examination showed clear evidence

/that

that there was an obstruction to the outflow of indian ink and therefore of aqueous humour into the episclera in all the glaucomatous eyes but not in the control eyes. In order to check this conclusion I showed the sections to Dr. Golda Selzer, of the Department of Pathology, University of Cape Town Medical School, who agreed with my interpretation.

In all eyes, glaucomatous and control, removed two hours after the injection of indian ink one finds the anterior chamber filled with ink but no ink in the sclera or episclera. A photomicrograph of one section from such a glaucomatous eye and one from a control eye is reproduced in Figures 42 and 43 respectively.

Twenty four hours after the indian ink injection the picture had changed. Ink had now passed through Schlemm's canal into the scleral and in the case of control eyes, the episcleral plexus's (Figure 46). However, in none of the

/sections

sections from glaucomatous eyes could ink be found in the episclera. In most of these sections ink had reached the deeper layers of the sclera and only in one section could ink be seen in the superficial scleral layers (this eye, No. 5, had an intraocular pressure of 34.5 millimetres of mercury); as this was the farthest point that the ink reached in any of the sections from glaucomatous eyes, a photomicrograph of this section is reproduced in Figure 44. A section from another glaucomatous eye showing ink in the deeper scleral layers is reproduced in Figure 45.

In control eyes on the other hand, ink had already reached the episclera after 24 hours. The typical appearance is shown in Figure 46 which is a photomicrograph of a section from a control eye removed 24 hours after injection of indian ink.

PATHOGENESIS :

Summarizing the result of this study it is evident the outflow channels from the anterior

/chamber

chamber through the sclera and episclera are obstructed in the glaucomatous rabbit eyes when compared to control eyes. In one section from a glaucomatous eye (Figure 44) indian ink had reached the superficial scleral layers, but in none of these sections had it reached the episclera.

This suggests that the maximum obstruction to outflow from the anterior chamber occurred in the episclera and adjacent scleral tissue.

Earlier in this thesis I pointed out (page 100) that the type of glaucoma induced in these experiments was an outflow glaucoma i.e. the ocular hypertension in the glaucomatous rabbit eyes was a direct result of an obstruction to the outflow of aqueous humour and was not due to hypersecretion of aqueous humour. This conclusion was based on the reported results of tonography (Table I, page 94) which demonstrated increased resistance to outflow of aqueous humour in glaucomatous as compared to control eyes (low

"C" values in the glaucoma eyes) and the results of the fluorescein appearance time test (Table 11, page 97) which indicated a reduced aqueous humour inflow in glaucomatous eyes.

This clinical evidence of obstruction to aqueous humour outflow in the glaucomatous rabbit eyes has been confirmed histologically. The histology has also shown that the maximum obstruction to outflow from the anterior chamber occurred in the episcleral and adjacent scleral tissue.

On the basis of this clinical and histological evidence one feels one can postulate that the subconjunctival injection of phenol in almond oil in the rabbit eyes so treated induced a change in the episcleral and adjacent scleral tissues which cannot be assessed morbid anatomically but nevertheless resulted in obstruction to the outflow of aqueous humour from the anterior chamber. Ultimately the degree of obstruction reached a level where it caused a gradual and sustained pathological rise in the intraocular pressure. This took, on an average,

/from

from four to eight weeks from the time of the first subconjunctival injection to reach an intraocular pressure of 30 millimetres of mercury.

At the same time aqueous humour inflow in glaucomatous eyes was significantly reduced when compared to control eyes, as measured by the fluorescein appearance time test. This probably represents a compensatory mechanism for the increasing obstruction to aqueous humour outflow and may account for the four to eight weeks delay between the first subconjunctival injection of phenol in almond oil and the first detected pathological rise of the intraocular pressure.

The exact nature of the change induced in the episcleral and scleral tissues in the glaucomatous eyes remains in doubt. Histology has failed to demonstrate any significant difference in the episclera and sclera of the glaucomatous and control eyes. Whatever the lesion, it is almost certainly diffuse, because

glaucoma could only be induced in eyes which had been injected in no fewer than all four quadrants. It is possible that the pathological changes are minimal and that sufficient obstruction to aqueous humour outflow to produce glaucoma occurs only when the lesion is so diffuse that it involves the entire circumference of the eye. I have already referred to Dr. Robb-Smith's opinion (page 109) that minimal changes in the episclera would be difficult to demonstrate by ordinary histological techniques.

This is well illustrated by a comparison of the reports submitted to me by two such eminent authorities as Dr. Robb-Smith and Dr. Michael J. Hogan, after they had examined sections from glaucomatous and control rabbit eyes. This they did "blindly" i.e. they were unaware at the time whether the slide they were examining was from a glaucomateus or control eye.

/Dr.

Dr. Robb-Smith reported that he could find no pathological change in any of the slides he examined. Dr. Hogan reported seeing "definite fibrosis" in the episclera of a number of the sections. On analysis it was found that these were sections from both glaucomatous and control eyes.

I have not been able to settle this point conclusively and it seems an impossible task to do so with the histological methods available at present. It is of some interest to point out that there is a similarity between this difficulty and what one finds in human primary open angle glaucoma. It has been shown by tonography and aqueous inflow studies that this is an outflow glaucoma, similar to the type of glaucoma induced in these rabbits. Yet persistent attempts to demonstrate significant pathological change in the outflow channels of human eyes with primary open angle glaucoma have been unsuccessful.

Although I have been unable to demonstrate

/any

any pathological change in the rabbit eyes with glaucoma, I feel it is permissable and indeed desirable to speculate on the possible nature of this change. I have shown that there is obstruction to aqueous humour outflow which is maximal in the episclera and adjacent sclera and which follows the injection of phenol into the episclera. I have earlier in this thesis (Section IV, page 30) pointed out that the major outflow of aqueous humour from the rabbit eye takes place, as it does in man, through the anterior chamber angle and via the scleral venous plexus's to the episcleral plexus's where it continues into the general circulation. Hence any pathology in the glaucomatous rabbit eyes causing obstruction to aqueous humour outflow must impede the circulation of aqueous in these channels, either by venous thrombosis or by obliteration of capillary plexus's secondary to diffuse fibrosis of the episcleral and scleral tissue. In my view it is unlikely, although possible, that venous thrombosis in the episcleral capillaries would remain localized to

/the

the smallest capillary plexus's in the episclera and adjacent sclera and escape histological detection. One would expect thrombosis occurring as a primary change following subconjunctival injection of phenol to spread and involve the larger vessels as well as the smallest and to be demonstrable on histology.

It seems more probable that the injection of phenol has caused a minor but diffuse inflammatory reaction in the episclera and adjacent scleral tissue. This has resulted in a minimal degree of fibrosis, not detectable by ordinary histological methods, which has resulted in obliteration or sclerosis of the smallest of the capillary plexus's in the episclera and adjacent sclera, occurring diffusely around the circumference of the eye. The pathology would not be due to any special property of the phenol used for injection, but merely the consequence of chemical irritation of the tissues.

In summary then, it is postulated that

/chemical

chemical irritation of the episcleral and adjacent scleral tissue in rabbit eyes injected subconjunctivally with phenol in almond oil causes diffuse but minimal fibrosis of these tissues around the circumference of the eye. This results in obliteration of the smallest episcleral and scleral capillary plexus's, so obstructing the outflow of aqueous humour from the anterior chamber of the eye. Ultimately this obstruction becomes severe enough, in most of the eyes so treated, to cause a pathological rise of the intraocular pressure, a pathological increase in the resistance to aqueous humour outflow and reduced inflow of aqueous humour into the eye, possibly as a compensatory mechanism for increased resistance to aqueous humour outflow.

That there is obstruction to aqueous outflow at the episclera and adjacent portion of the sclera in the rabbit eyes with glaucoma is clear from the evidence that I have presented .

/That

That this obstruction is the result of episcleral and scleral fibrosis with obliteration of the smallest capillary plexus's is speculative.

- - - - - - - - - - - - - - - -

SECTION IX

S U M M A R Y

A method of producing primary glaucoma in rabbits by means of the subconjunctival injection of phenol is described. In nine out of ten rabbits treated in this way, a chronic glaucoma (as described in the introduction) resulted. The effects of Guttae Pilocarpine and Diamox were studied in two rabbits, and of iris inclusion operations on four eyes, two control and two treated.

Aqueous outflow and inflow studies suggest that the inflow of aqueous was reduced and the resistance to outflow increased in treated eyes with raised intraocular pressure.

The methods have been described in detail, and a critical analysis of the validity of the results has been offered.

Most of the animals used in these

/experiments

experiments were killed and their eyes removed for histology.

The outflow channels from the anterior chamber angle of a further eight glaucomatous and six normal rabbit eyes were studied histologically after an injection of indian ink into the anterior chamber.

The pathogenesis of this type of experimental glaucoma is discussed in relation to the clinical and histological findings.

- - - - - - - - - - - - - - - - -

SECTION X

R E F E R E N C E S

1) Ascher, K.W. (1951). "XVIConc. Ophthal. 1950" Britannia Acta., vol. 1, pp. 674-687. B.M.A., London.

2) Ascher, K.W. (1952). Amer. J. Ophthal. 35, 5, part 2, p. 10.

3) Ashton, N. (1951). Brit. J. Ophthal. 35, p. 291.

4) Ashton, N. (1952). Ibid, 38, p. 129.

5) Ashton, N. (1955). Glaucoma Symposium (Council for International Organization of Medical Sciences) edited by Stewart Duke-Elder, Blackwell Scientific Publications, Oxford, England.

6) Bain, W.E.S. (1954). Brit. J. Ophthal. 38, p. 129.

7) Barkan, O. (1954). Amer. J. Ophthal. 37, p. 724.

8) Becker, B. and Constant, M. (1956). A.M.A. Arch. Ophthal. 55, pp. 305-312.

/9)

9) Bentzen, Chr. F. (1895). V. Graefes Arch. Ophthal. 41, 4 p. 42.

10) Berggren, L. (1956). Amer. J. Ophthal. 42, p. 595.

11) Davis, F.A. (1929). Trans. Amer. Ophthal. Soc. 27, p. 401.

12) Dobree, J.H. (1953). Brit. J. Ophthal. 37, p. 293.

13) Dvorak-Theobald, G. (1934). Trans. Amer. Ophthal. Soc., 32, p. 574.

14) Flocks, M., Tsukahara, I., Miller, J. (1959). Amer. J. Ophthal. 48, 1, pt.2 p. 11.

15) Friedenwald, J.S. (1937). Amer. J. Ophthal. 20, p. 985.

16) Friedenwald, J.S. (1950). Amer. J. Ophthal. 33, p. 1523.

17) Friedenwald, J.S. (1954). "Calibration of Tonometers". Decennial Report by the Committee on Standardization of Tonometers. Amer. Acad. Ophthal.

18) Friedenwald, J.S. (1957). "Tonometer Calibration". Trans. Amer. Acad. Ophthal. and Otolaryng. 61, p. 108.

/19)

19) Fuchs, H.E. (1923). "Textbook of Ophthalmology", 7th ed. pp. 498-612. Lippincott, London.

20) Goldmann, H. (1946). Ophthalmologica (Basel), III. P. 146.

21) Grant, W.M. (1950). A.M.A. Arch. Ophthal. 44, p. 204.

22) Grant, W.M. and Trotter, R.R. (1955). A.M.A. Arch. Ophthal. 53, p. 191.

23) Grant, W.M. (1955). A.M.A. Arch. Ophthal. 54, p. 245.

24) Grant, W.M. (1958). A.M.A. Arch. Ophthal. 60, p. 523.

25) Heisrath, F. (1879). Centralblad. med. Wiss. 43, p. 769.

26) Huggert, A. (1951). Acta Ophthalmologica. (kbl). 29, p. 474.

27) Huggert, A. (1957). Acta Ophthalmologica. 35, 1, p. 1.

28) Kajigaya, Y. (1951). Acta Soc. Ophthal. Japan 55, p. 867. Cited from Ophthal. Lit. 5, p. 5239. (1951).

29) Kornblüth, W. and Linnér, E. (1955). A.M.A. Arch. Ophthal. 54., p. 717.

30) Koster, W. (1895). V. Graefes Arch. Ophthal 41, 2., p. 30.

31) Leber, T. (1873). V. Graefes Arch. Ophthal. 19, pt. 2., p. 87.

32) Levene, R.Z. and Bloomgarden, C. (1961). A.M.A. Arch. Ophthal. 66, 2. p. 149.

33) Linnér, E. and Friedenwald, J.S. (1957). Amer. J. Ophthal. 44, pt. 2, p. 225.

34) Luntz, M.H. and Smith, Redmond. (1960). Brit. J. Ophthal. 44, 10. p. 600.

35) Maggiore, L. (1917). Ann. Ottal. Clin. Ocul. 40., p. 317.

36) Nemetz, U.R. (1956) Ber. dtsch. Ophthal. Ges. 60., p. 60.

37) Pickering, G.W. (1955). "High Blood Pressure". p. 85. J. and A. Churchill Ltd., London.

38) Ruskell, G.L. (1961). A.M.A. Arch. Ophthal. 66., p. 861.

39) Salzmann, M. (1912). "Anatomie und Histologie dens menslichen Augappels in Normalzustande". Leipzig und Wein. F. Deuticke. Translated by E.V.L. Brown, University of Chicago Press, 1912.

40) Sampson, G.S. and Girard, L.J. (1961). Amer. J. Ophthal. 52, pt. 2. p. 5.

41) Schlemm, F. (1831). Z. Ophthal. 1, pt. 1. p. 113.

42) Schöler, A. (1879). V. Graefes Arch. Ophthal, 25, 4, p. 63.

43) Seidel, E. (1918). Arch. f. Ophthal. 95. p. 1.

44) Smith, Priestley. (1891). "On the Pathology and Treatment of Glaucoma". Churchill, London.

45) Speakman, J. (1960). Brit. J. Ophthal. 44, p. 513.

46) Thomassen, T.L. (1948). Trans. Ophthal. Soc. U.K. 68, p. 75.

47) Tuovinen, E. (1961). Acta Ophthalmologica, Supplement 67.

48) Weber, A. (1877). V. Graefes. Arch. Ophthal. 23, pt. 1., p. 1.

49) Weekers, L. (1921). Arch. Internat. Physiol, 18., pp. 200-212.

50) Weekers, R. and Prigott, E. (1950). Ophthalmologica 119., p. 321.